Virchows Archiv (1994) 425:399–406

ORIGINAL ARTICLE

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Gene expression of CD24 core polypeptide molecule in normal rat tissues and human tumor cell lines

Received: 8 March 1994 / Accepted: 27 June 1994

Abstract CD24 antigen is a glycoprotein expressed on haematopoietic cells, including B cells, T cells and granulocytes and on non-haematopoietic cells, including neural cells, ganglion cells and the cells of the adrenal medulla. The antigen is also expressed on renal cell carcinoma, small cell lung carcinoma and neuroblastoma. We have cloned rat cDNA encoding core polypeptide of CD24 antigen from embryonic brain and shown that the core molecule is highly expressed in embryonic brain and non-neural tissues. Rat tissue and various human neoplastic cell lines were investigated for the gene expression of CD24 core polypeptide by in situ hybridization. The transcript was localized in gastrointestinal epithelia, ductal and acinar epithelia of the salivary gland, the bronchiolar epithelium, renal tubular epithelium, the epithelium of the oviduct, follicular cells of the thyroid, medullary cells of the adrenal gland, Auerbach's plexus, B blastoid cells in lymph nodes, hair follicles, and the sweat glands of the skin. Among the various human neoplastic cell lines investigated, the transcript was detected in squamous cell carcinoma of the lung, gastric carcinoma, colon carcinoma, choriocarcinoma and renal cell carcinoma. The result suggest that the core molecule of CD24 antigen may be expressed in a wider range of epithelial cells and carcinoma cell lines than has been reported. Furthermore, we show that gene expression of CD24 core polypeptide is confined to the proliferative zone of the gastrointestinal mucosa, suggesting that core molecule is transiently expressed on the surface of epithelial cells in the process of cellular maturation. We discuss a possible role for CD24 antigen in the maturation of epithelial cells in the gastrointestinal tract.

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Key words CD24 · Core molecule
Gastrointestinal stem cell · In situ hybridization
Neoplastic cell line

Introduction

CD24 antigen is a glycoprotein expressed on haematopoietic cells (B cells, T cells, and granulocytes; [1, 5, 7, 15, 19]). Recently, it was revealed that CD24 antigen was also expressed in some non-haematopoietic cell lineages including neural cells, ganglion cells and adrenal medullary cells [18]. The antigen is expressed in renal cell carcinoma (RCC) and tumour cells of neural origin, such as neuroblastoma or small cell lung carcinoma [8, 13, 14]. In small cell lung carcinoma, CD24 antigen is one of the major surface glycoproteins and is currently expected to be a target of effective immunotherapy [11]. Molecular cloning of murine, human and rat cDNA encoding core polypeptide of CD24 antigen revealed that CD24 antigen is a heavily glycosylated membrane protein anchored by GPI linker [12, 13, 23]. However, little is known of the function of the CD24 antigen. One of the monoclonal antibodies against CD24 antigen, OKB2, can block the differentiation of resting B cells, suggesting a possible role of CD24 antigen on differentiation of B cells [20]. In another study, cross-linking of CD24 antigen on the surfaces of B cells by monoclonal antibody resulted in a rapid rise in cytoplasmic calcium levels, suggesting that CD24 antigen can exert its effects on B cells by initiating a calcium-mobilizing signal transduction pathway [9].

In a previous paper, we showed that one of the developmental brain-characteristic genes turned out to encode the core molecule of rat CD24 antigen [23]. We characterized the gene expression in rat embryo by in situ hybridization, demonstrating that the gene of CD24 core polypeptide is highly transcribed in developmental brain and developmental non-neural tissues such as intestinal mucosa, nasal mucosa the ductal epithelium of the salivary glands, bronchial epithelium, renal tubular epithelium, in developing teeth and developing hair follicles.

Previous study indicated two lines of evidence implying a putative roles for CD24 antigen in developing embryo. The first one is that the gene encoding CD24 core molecule is specifically transcribed in postmitotic neuronal population in the neuroepithelium of the developing brain and may be involved in cell migration [17, 23]. The other is that in the developing tooth this molecule is specifically induced in differentiating odontoblasts of the dental papilla and may be involved in the cell-to-cell interaction or cell-to-matrix interaction which initiates the cellular differentiation of odontoblasts.

In this paper we investigated the gene expression of CD24 core molecule in rat adult tissues and various human neoplastic cell lines, revealing that the gene expression of CD24 core polypeptide is down-regulated in many tissues when organogenesis is completed. Nevertheless, CD24 core polypeptide gene is still strongly expressed in some specific cells in gastrointestinal tract, kidney, oviduct, and grandular organs such as salivary gland, thyroid gland, and prostate gland in adult tissues. Gene expression of CD24 core polypeptide is observed here in some neoplastic cell lines including adenocarcinoma of gastrointestinal tract, squamous cell carcinoma of the lung and choriocarcinoma. We have also identified the cells expressing the gene of CD24 core polypeptide in the proliferative zone of gastrointestinal mucosa by in situ hybridization and bromodeoxyuridine (BrdU) immunohistochemistry, and discussed the putative function of CD24 molecule in the maturation of gastrointestinal epithelium.

Materials and methods

Human neoplastic cell lines were provided by the Japanese Cancer Research Resources Bank, Tokyo: YMB-1-C (human breast cancer), EBC-1 (human lung squamous cell carcinoma), RERF-LC-OK (human lung adenocarcinoma), HSC-2 (human oral squamous cell carcinoma), NUGC-2 (human gastric cancer), COLO201 (human colonic cancer), HepG2 (human hepatoma), MIA PaCa-2 (human pancreatic cancer), MCAS (human ovarian mucinous cystadenocarcinoma), Hela (human uterine cervical epithelioid carcinoma), Bewo (human choriocarcinoma), ITO-II (human testicular tumour), VMRC-RCW (human RCC of proximal origin) and VMRC-RCZ (human RCC). These cells were cultured in Dulbecco's Modified Eagle's Medium (GIBCO, Grand Island, New York) containing a recommended concentration of fetal calf serum.

Total RNAs were extracted from various cell lines using a single step method of acid guanidine thiocyanate phenol chloroform method [6]. Messenger RNAs were isolated from various rat tissues as described previously [23]. Ten micrograms of total RNA or 1 μg of poly $(A)^+$ RNA were electrophoresed on 1.2% agarose 10% (v/v) formaldehyde gel, transferred to nylon membranes (Hybond N^+; Amersham International, UK) and hybridized to ^{32}P-labelled probes. Then, 1 kb *Eco* RI fragment (nucleotides 1–1,047) of rat CD24 cDNA clone was labelled by the random priming method (Boehringer Mannheim, Germany) and used as hybridization probe. Hybridization was performed in 4× SSC, 10× Denhardt, 1% SDS, 50 mM TRIS-HCl pH 7.6, 10 mM EDTA, 5% dextran sulphate, 100 μg/ml sonicated herring sperm DNA, 100 μg/ml poly (A) at 65° C for 16 h. Filters were washed at 65° C in 0.5× SSC and 0.5% SDS, exposed at −70° C to Kodak XAR-5 film with an intensifying screen. Rat EF-1α cDNA probe or mouse β-actin cDNA probe was used for control hybridizations [22, 24].

For in situ hybridization adult rat tissues were fixed in 4% paraformaldehyde in phosphate buffered saline (PBS) at 4° C overnight, dehydrated with ethanol, and embedded in paraffin. Serial sections were cut at 5 μm and mounted on poly-L-lysine (Sigma, St. Louis, Mo.) coated slides. After removal of wax, sections were postfixed in 4% paraformaldehyde, treated with 0.25% acetic anhydrite in 0.1 M triethanolamine, and dehydrated again. For antisense probe pBluescript plasmid containing full-length rat CD24 was linearized with *Xba* I and transcribed by T3 RNA polymerase according to the manufacturer's recommendations (Promega, Madison, Wis.) with ^{35}S-UTP (>1000 $Cimmol^{-1}$; Amersham, UK). Sense probes were synthesized by T7 RNA polymerase after linearization of the plasmid with *Bam* HI. Hybridizations were performed at 50° C for 16 h in 50% deionized formamide, 10 mM TRIS-HCl pH 7.6, 1 mM EDTA, 600 mM NaCl, 0.25% SDS, 1 × Denhardt's solution, 10% dextran sulphate, 10 mM DTT, 200 μg/ml *E. coli* tRNA, and 1×10^7 cpm/ml radiolabelled probe. After hybridization, samples were treated with 50 μg/ml RNase A at 37° C for 30 min. Washing was performed in 0.1 × SSC at 50° C. Slides were then dehydrated with ethanol. Autoradiography was performed using Kodak NTB-3 emulsion diluted 1:1 with 2% glycerol in distilled water. After exposure, slides were developed using Kodak D-19 and fixed by Fujifix. Sections were counterstained with toluidine blue.

Thirty milligrams of BrdU (Sigma) was administered intravenously to a 3-month-old rat for labelling of the gut. Thirty minutes after the injection, the rat was killed and tissues fixed in ethanol overnight prior to embedding in paraffin. Sections were cut at 4 μm. Incorporated BrdU was detected by indirect immunohistochemical staining using the peroxidase-antiperoxidase (PAP) complex method. Deparaffinized sections were treated with methanol solutions containing 0.3% (v/v) hydrogen peroxide for 30 min to inhibit endogenous peroxidase activity. After washing with PBS, sections were treated with 2 N hydrochloride for 30 min to degenerate DNA. Sections were neutralized with 0.1 M borate buffer pH 8.5 and were overlaid with PBS containing 10% normal rabbit serum for 15 min to inhibit non-specific absorption of primary antibody. Sections were sequentially treated with anti-BrdU monoclonal antibody (Becton-Dickinson, Mountain View, Calif. × 50), peroxidase-conjugated rabbit anti-mouse IgG (Dako Japan, Kyoto, × 50) and PAP (Dako Japan, Kyoto, × 50). The enzymatic products of peroxidase activity were developed with 0.03% diaminobenzidine (Sigma) in 50 mM TRIS-HCl, pH 7.6 containing 0.03% hydrogen peroxide.

Results

Gene expression of CD24 core polypeptide in normal adult rat tissues

The gene regulation of CD24 core polypeptide in normal tissues or various neoplastic cell lines has not been investigated and so we initially studied gene expression of CD24 core polypeptide in various normal rat tissues by RNA blot analysis. As shown in Fig. 1, the gene expression was detected in rat salivary gland, kidney, ovary, bone marrow, spleen, thymus and muscle. Faint but distinct signal was detectable in lung, adrenal gland and brain. No gene expression was detected, even by long exposure, in testis, liver and heart. Compared with the embryonic pattern, CD24 core polypeptide was transcriptionally down-regulated in adult brain, thymus and lung [23], whereas gene expression remained high in adult salivary gland and kidney. Interestingly, RNA blot analysis revealed that gene expression of CD24 core polypeptide was found in such tissues as salivary gland

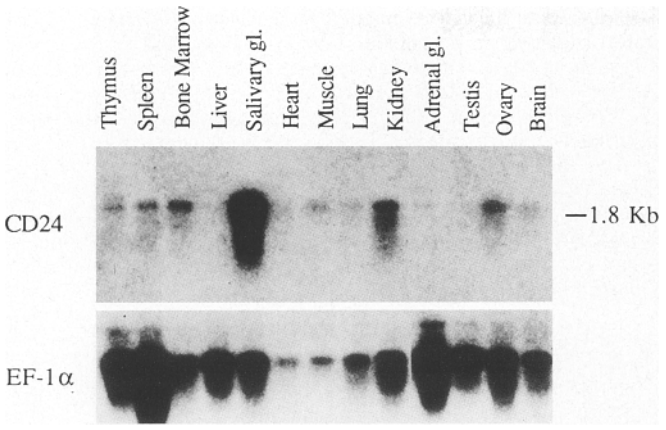

Fig. 1 Northern blot analysis of RNAs isolated from thymus, spleen, bone marrow, liver, salivary gland, heart, muscle, lung, kidney, adrenal gland, testis, ovary and brain of adult Wistar rat. One microgram of poly(A)$^+$ RNA *(upper panel)* or 0.2 µg of poly(A)$^+$ RNA *(lower panel)* was separated on 1.2% agarose gel containing 10% formaldehyde and blotted onto Hybond N$^+$ nylon membrane. The blots were hybridized with 1.0 kb *Eco* RI fragment of rat CD24 cDNA *(upper panel)* or rat EF-1α probe *(lower panel)*. The molecular size of the hybridizing signal was estimated using RNA molecular size marker (Boehringer Mannheim, Germany). Probes used are shown on the *left* and the size of the hybridizing signal on the *right*

Table 1 CD24 mRNA expressions in normal tissues

Tissue	Main hybridizing structures
Digestive Tract	
Stomach	Epithelium (neck)
Duodenum, jejunum	Epithelium (bottom of crypt)
Colon	Foveolar epithelium (lower part of crypt)
Digestive glands	
Salivary gland	Ductal epithelium, acinar epithelium
Liver	[a]
Pancreas	ND[b]
Respiratory system	
Lung	Bronchial and bronchiolar epithelium
Urinary system	
Kidney	Renal tubular epithelium
Ureter	ND
Bladder	ND
Reproductive system	
Uterus	Endometrial epithelium
Ovary	Corpus luteum
Oviduct	Tubal epithelium
Prostate	Glandular epithelium
Endocrine system	
Thyroid	Follicular cells
Adrenal	Medullary cells
Nervous system	
Brain	Neurons
Peripheral nerves	Auerbach's plexus
Vascular system	
Heart	–
Blood vessels	–
Immune system	
Thymus	Mucous cells
Spleen	–
Lymph nodes	Germinal centres
Others	
Skin	Hair follicles, sweat glands
Eye	Sclera

[a] No signal detected
[b] Not determined

or ovary where CD24 antigen molecule could not be detected by immunohistochemical investigations. To address this issue further, we studied the localization of the transcript of CD24 core polypeptide by in situ hybridization and identified the transcription-positive cells in various rat normal tissues. The results are summarized in Table 1.

In the gastrointestinal tract, gene expression of CD24 core molecule was detected in a specific population of cells in gastric, small intestinal and large intestinal mucosa (Fig. 2). In fundic mucosa of the stomach, the hybridization signals of CD24 core polypeptide were most pronounced in the upper-middle layer of gastric mucosa, but were less intense in other layers (Fig. 2C). In pyloric mucosa of the stomach, however, the hybridization signals were localized to the lower-middle layer of gastric mucosa (Fig. 2F). In addition, the specific expression of CD24 core polypeptide was slightly detected in the bottom portion of small intestinal crypts (Fig. 2I) and heavily in large intestinal crypts (Fig. 3A). Based on the distribution of transcription-positive cells in the gastrointestinal tract, we hypothesized that the epithelial cells expressing CD24 core polypeptide gene correspond either to gastrointestinal stem cells or upward-migrating epithelial cells which have just differentiated from the stem cells. To test this hypothesis we compared the immunohistochemical localization of BrdU incorporation with that of CD24 transcription-positive cells by in situ hybridization (Fig. 2A–I). The localization of hybridization signals in the fundic mucosa of the stomach (Fig. 2B), the pyloric mucosa of the stomach (Fig. 2E) and the small intestine (Fig. 2H) corresponded well to that of BrdU-positive cells (Fig. 2C, bright field; F, I, dark field). These findings strongly suggest that BrdU-positive gastrointestinal stem cells or the postmitotic cells just differentiated from stem cells may express CD24 core polypeptide gene and that they may lose gene expression of CD24 core polypeptide during their upward migration.

In the submandibular gland, the strong hybridization signals of CD24 core polypeptide were detected throughout the gland (Fig. 3E) including ductal epithelium and acinar cells of both mucous and serous glands. In the kidney, some cortical and medullary tubular structures showed specific hybridization (Fig. 3F). These signals contrasted with the negative hybridization in the interstitial connective tissue of the kidney. In glomeruli, mesangial cells and the visceral layer of Bowman's capsule were all negative. The pattern of hybridization signals in renal tubular structures suggests mRNA localization of

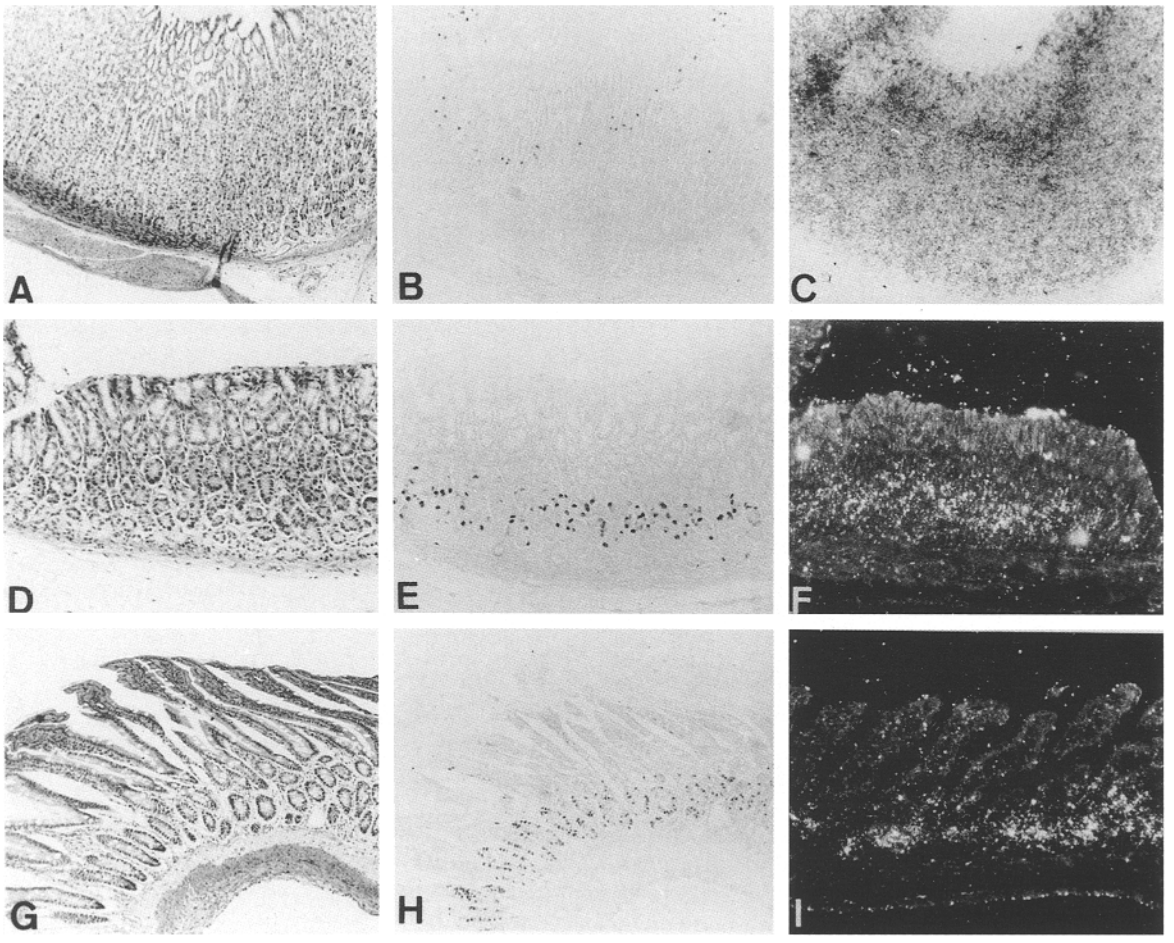

Fig. 2A–I Correlation between the proliferative activity of gastrointestinal epithelium and CD24 gene expression. Upper (**A–C**), middle (**D–F**) and lower columns (**G–I**) show fundic mucosa, pyloric mucosa of the stomach and jejunal mucosa, respectively. Left, medial and right rows show H&E staining, immunohistochemical localization of incorporated bromodeoxyuridine (BrdU) and CD24 gene expression analysed by in situ hybridization (**C,** bright field; **F, I,** dark field), respectively. CD24 gene expression was strongly detected in the upper-middle layer of the fundic mucosa (**C**), in the lower-middle layer of the pyloric mucosa (**F**), and in the bottom of the jejunal crypts (**I**). The localization of hybridization signals in each of the mucosa corresponded well to that of BrdU-positive cells (**B, E, H**). **A–C,** ×50; **D–F,** ×100; **G–I;** ×60

CD24 core polypeptide in distal and collecting tubules rather than in proximal tubules (Fig. 3F). This pattern of expression was comparable to the developing kidney in which proximal tubules and subcortical glomeruli showed specific hybridization [23]. In genitourinary tissues, strong hybridization signals were found in the tubal epithelium of the oviduct (Fig. 3B) and less intense signals in the endometrium, comparable with the negative hybridization in the interstitial tissue of the oviduct and the myometrium of the uterus. Since the hybridization signals in fimbriae were much stronger than those in the ovary (Fig. 3B), the intensity noted in RNA blot analysis may be due to the gene expression of CD24 core polypeptide in the oviduct. In the prostate, CD24 core polypeptide was expressed in the basal layer of the glands in which a variable degree of gene expression was found from cell to cell (Fig. 3D). In endocrine tissues, CD24 core polypeptide was expressed in the follicular cells of the thyroid gland (Fig. 3G) and in the medullary cells of the adrenal gland (Fig. 3H). No apparent hybridization signal was found in cortical cells of the adrenal gland. In haematopoietic tissues, patchy hybridization signals were found in the thymus, while most thymocytes and stromal cells were negative for the gene expression of CD24 core polypeptide (data not shown). These signals may correspond to mucous cells judging from the localization and the frequency of hybridization signals, although further studies would be needed to confirm this. In lymph nodes, scattered hybridization signals with moderate intensity were noted specifically in the germinal centre, while small lymphocytes and other types of cells in outer area of the germinal centre were all negative (data not shown). These hybridization signals may correspond to mature or blastoid B cells. In skin, CD24 core polypeptide was expressed in hair follicles and sebaceous glands (Fig. 3C). Striated muscle, heart muscle, and smooth muscle, connective tissues, chondrocytes, cartilage, testis, and blood vessels were generally negative.

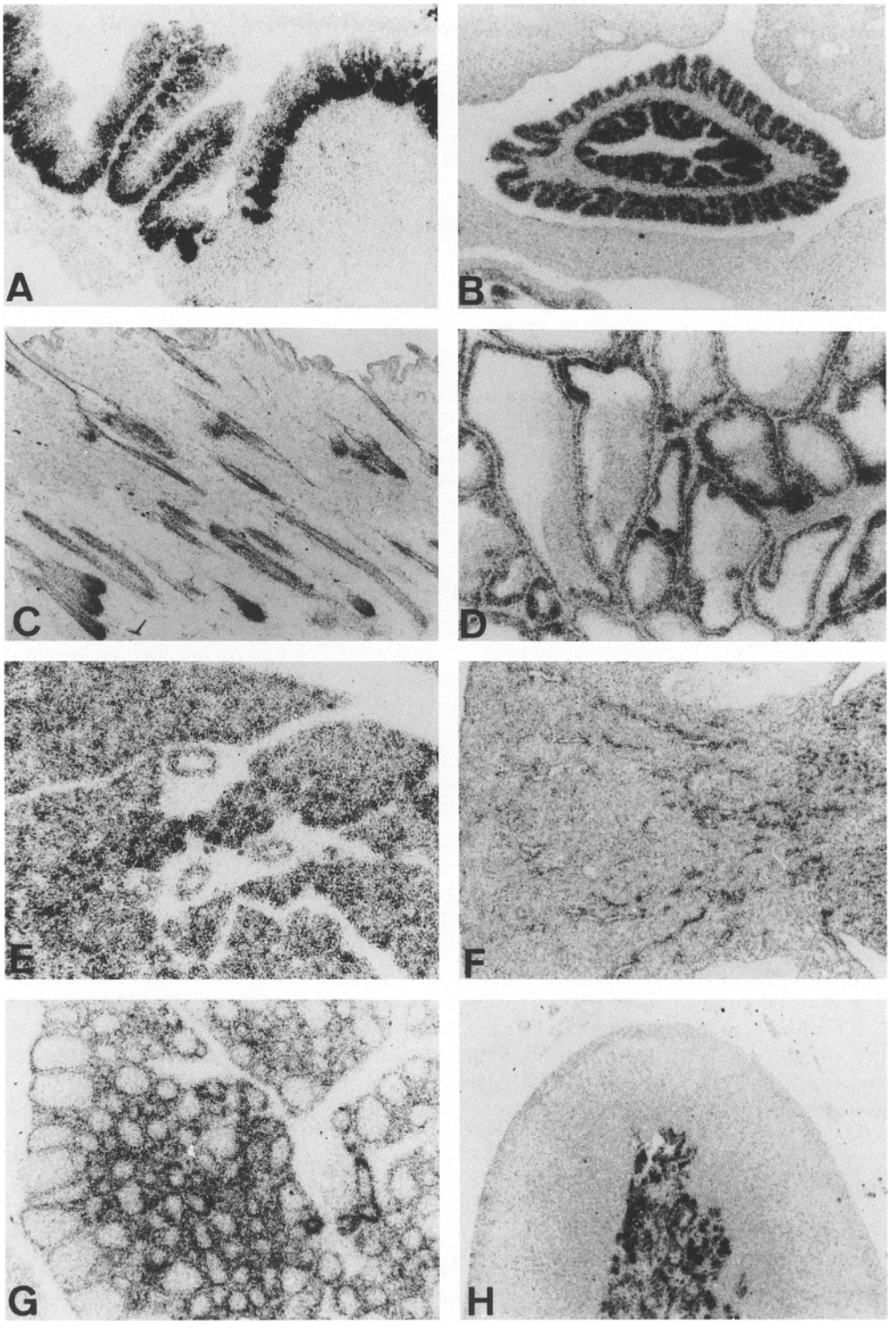

Fig. 3A–H CD24 gene expression in various tissues of adult Wistar rat analysed by in situ hybridization. CD24 gene was expressed in the crypt bottom of the large intestine (**A**), in the epithelium of the oviduct including the rolling back-portion at the fimbriae (**B**), in the matrix cells of both the hair root and hair shaft of the skin (**C**), in the grandular epithelium of the prostate gland (**D**), in both ductal and acinar epithelium of the submandibular gland (**E**), in some part of the cortical and medullary tubules but not in the glomerulus of the kidney (**F**), in the follicular cells of the thyroid gland (**G**) and in the medullary cells but not in the cortical cells of the adrenal gland (**H**). **A,** ×18; **B,** ×40; **C,** ×33; **D,** ×50; **E,** ×80; **F,** ×40; **G,** ×60; **H,** ×30

Expression of CD24 core polypeptide molecule in human tumor cell lines

We investigated the gene expression of CD24 core polypeptide in various tumour cell lines (Fig. 4) including breast carcinoma (YMB-1-C), squamous cell carcinoma of the lung (EBC-1), adenocarcinoma of the lung (RERF-LC-OK), squamous cell carcinoma of the mouth (HSC-2), gastric carcinoma (NUGC-2), colonic carcinoma (COLO201), hepatoma (HepG2), pancreatic carcinoma (MIA PaCa-2), ovarian mucinous cystadenocarcinoma (MCAS), uterine cervical epithelioid carcinoma (Hela), choriocarcinoma (Bewo), embryonal carcinoma of the testis (ITO-II) and RCC (VMRC-RCZ and

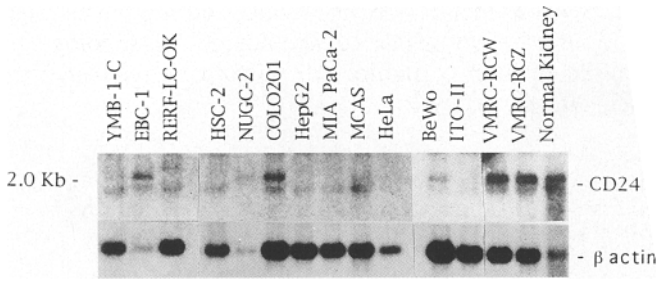

Fig. 4 Northern blot analysis of RNA isolated from various tumour cell lines and normal human kidney tissues. Ten micrograms of total RNAs were separated on 1.2% formaldehyde gel and blotted onto Hybond N^+ nylon membrane. The blots were hybridized with 1.0 kb *Eco* RI fragment of rat CD24 cDNA *(upper panel)* or mouse β-actin probe as a control hybridization. Total RNAs were extracted from various types of human tumour cells in culture, YMB-1-C (human breast cancer), EBC-1 (human lung squamous cell carcinoma), RERF-LC-OK (human lung adenocarcinoma), HSC-2 (human oral squamous cell carcinoma), NUGC-2 (human gastric cancer), COLO201 (human colonic cancer), HepG2 (human hepatoma), MIA PaCa-2 (human pancreatic cancer), MCAS (human ovarian mucinous cystadenocarcinoma), Hela (human uterine cervical epithelioid carcinoma), Bewo (human choriocarcinoma), ITO-II (human testicular tumour), VMRC-RCW and VMRC-RCZ (human renal cell carcinoma)

VMRC-RCW) as well as normal human kidney tissue. As shown in Fig. 4, a strong hybridization signal of 2.0 kb was detected in squamous cell carcinoma of the lung, colonic carcinoma, renal cell carcinoma; less intense but distinct signals were detected in gastric carcinoma and choriocarcinoma. In these carcinoma cell lines, gene probe of rat CD24 core polypeptide specifically detected a 2.0 kb human transcript. Although this transcript is a little longer than rat homologue, this may be due to a species difference. In NUGC-2, gastric carcinoma cell line, the amount of RNA mounted on the filter was so limited judging from the intensity of β-actin hybridization that the equivalent amount of CD24 core polypeptide may be expressed in gastric cancer as compared with that in colonic cancer (Fig. 4, lanes 5 and 6). We could not detect the 2.0 kb transcript of CD24 core polypeptide in other cancer cell lines.

Discussion

We have investigated the gene expression of CD24 core polypeptide in normal tissues and various human tumour cell lines and show that the gene encoding CD24 core polypeptide is widely transcribed in various tissues including salivary gland, kidney, gastrointestinal tract, oviduct, adrenal gland, respiratory bronchiole, thyroid gland, prostate gland, hair follicle and haematopoietic cells. Gene expression is also detected in tumour cell lines such as squamous cell carcinoma of lung, gastric carcinoma, colonic carcinoma and choriocarcinoma.

Expression in normal rat tissues (adrenal medullary cells, peripheral nervous tissue) is consistent with previously reported distributions of CD24 antigen in normal human tissues investigated by monoclonal antibody [18]. In the medulla of the adrenal gland, strong in situ hybridization signals are not in good agreement with a faint signal in the Northern blot analysis (Figs. 1, 3H). The discrepancy in the results may be due to the different probes used in these analyses: the probe used in Northern blot analysis lacked 400 bp in the 3′ region of cDNA. Alternatively spliced mRNA may then explain the stronger in situ signals of the adrenal gland; however, further study would be needed to define this.

The gene expression of CD24 core polypeptide in salivary gland, gastrointestinal tract, oviduct, respiratory bronchiole, and thyroid gland as well as various tumour cell lines (gastric carcinoma, colonic carcinoma, and choriocarcinoma) has not yet been reported in spite of the extensive investigations [3, 8]. A possible explanation for this may be the discrepancy between the expression of CD24 core polypeptide and the post-translational modification of CD24 antigen sugar structure, which often provides the major epitopes recognized by the monoclonal antibodies [16].

In the gastrointestinal tract, in situ hybridization study showed that the transcript was specifically localized to the proliferative zone in the gastrointestinal mucosa and that its localization was similar to the distribution of epithelial stem cells labelled with BrdU. Thus, CD24 core polypeptide transcription-positive cells may either correspond to BrdU-positive gastrointestinal stem cells or to postmitotic epithelial cells in their early stage of maturation. Jackson and colleagues [11] argued that CD24 antigen may be the surface receptor for cell proliferation especially in small cell lung carcinoma. In the light of this hypothesis, it is intriguing to speculate that CD24 core polypeptide expressed in the gastrointestinal stem cells may relate to the cellular proliferation rather than to cellular differentiation. In contrast, our previous study of embryo showed that gene expression of CD24 core polypeptide is restricted to the postmitotic cell population in developmental neuroepithelium, where the postmitotic neuronal population is easily distinguished from stem cells microscopically [23]. By analogy, in the maturation process of gastrointestinal epithelial cells, the cells may involve gene expression of CD24 core polypeptide immediately after the mitosis, with maintenance of expression during the early stage of maturation and loss in the course of maturation.

In the gastrointestinal carcinoma cell lines investigated, gastric cancer (NUGC-2) and colonic cancer (COLO201), the gene expression of CD24 core polypeptide was detected in RNA blot analysis. Since both gastric and colonic cancer are supposed to originate from the cells in the proliferative zone located in the neck region of gastric mucosa or in the lower region of intestinal crypts [4, 10], it is reasonable to assume that carcinoma cells of gastrointestinal origin express CD24 core polypeptide as a phenotypic marker of their origin.

In the early stage of cellular maturation, two possible functions of CD24 antigen can be suggested on the basis of common features between the developmental central

nervous system and the adult gastrointestinal tract [17, 23]. The first possibility is that CD24 molecule may function as a receptor for a certain lectin or lectin-like molecule, which can initiate the signal transduction on the differentiation of gastrointestinal epithelial cells. The second possibility is that CD24 may function as an adhesion molecule that supports the early migration of epithelial cells in the gastrointestinal mucosa, which is comparable to migrating granular cells in developing cerebellum.

In kidney development, strong expression of CD24 antigen in the primitive induced metanephric blastema, primitive nephron (vesicle and S-shaped body) were reported in previous immunohistochemical studies [8, 21]. The maturation of the proximal tubule, which derives from the intermediate part of the S-shaped body, is marked by an abrupt loss of CD24 antigen. At the same time, the upper part of the S-shaped body, the future distal tubule, retains CD24 antigen and later synthesized Tamm-Horsfall protein. In the adult kidney, CD24 antigen was restricted to the distal part of the nephron [21]. Our results using in situ hybridization show that the expression of CD24 core polypeptide in the developmental and adult kidney may essentially support the previous immunohistochemical study.

In neoplasia, Droz and colleagues [8] examined the CD24 expression in 28 cases of RCC including 21 clear, 1 eosinophilic, 4 basophilic, and 2 spindle-shaped cell types by immunohistochemistry. The results showed that all RCC cases except the spindle-shaped cell type highly expressed CD24 antigen. Borowitz and colleagues [3] also conducted a similar study on 20 cases of RCC and reported a similar result. Also in our Northern analysis, both of the RCC lines showed gene expression of CD24 core polypeptide. Since most of the RCC have been considered to arise from proximal tubular epithelium, the authors argued that RCC regained the expression of CD24 antigen in the process of oncogenesis as an oncofetal antigen by analogy with many other cancers. Alternatively, CD24 antigen may have some role in determining the differentiated phenotype of proximal renal tubular cells since anaplastic RCC does not express the antigen [8].

The role of CD24 antigen in adrenal medulla, thyroid gland, salivary gland, prostatic gland and oviduct (see Fig. 3B, D, E, G, and H) is poorly understood. In the developing embryo, however, CD24 core polypeptide is transcribed in almost all branching organs such as salivary glands, respiratory bronchi and pancreas [23]. We have discussed a role for the molecule in cell-to-cell interaction between epithelial cells and mesenchymal cells during the morphogenesis of branching structures in the developing embryo. In this respect, the epithelial-mesenchymal interaction via CD24 antigen may be required, even after organogenesis has been completed for the maintenance of branching structure in excretory glands such as salivary gland and prostatic gland. It is also intriguing to speculate that CD24 antigen may play a role in cell-to-cell interaction between epithelial cells and mesenchymal cells of the oviduct during the estrous cycle, since epithelial cells undergo morphological changes including ciliation, deciliation or regeneration during the sexual cycle [2].

Acknowledgements We thank Drs. M. Kitamura for RNA of renal cell line, Dr. K. Sakamoto and Mrs. E. Mori-izumi for technical assistance, and Drs. N. Maruyama and C. Kurashima for valuable discussions.

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