Cellular Localization of the Folate Receptor: Potential Role in Drug Toxicity and Folate Homeostasis

Steven D. Weitman, Arthur G. Weinberg, Leslie R. Coney, Vincent R. Zurawski, Debra S. Jennings, and Barton A. Kamen

Department of Pediatrics [S. D. W., A. G. W., B. A. K.], Pathology [A. G. W., D. S. J.], and Pharmacology [B. A. K.], University of Texas Southwestern Medical Center and Children’s Medical Center of Dallas, Dallas, Texas 75335, and Cancer Research Division, Centocor, Malvern, Pennsylvania 19355 [L. R. C., V. R. Z.]

Abstract

In the past year, gp38, a glycosyl-phosphatidylinositol linked membrane protein that is overexpressed in some malignant tissues, has been shown to be the folate receptor. Using immunohistochemical techniques with the monoclonal antibody MOv19 against gp38, we evaluated the cellular localization of folate receptors in normal human tissues, which are potential target sites for drugs that utilize this uptake mechanism. The choroid plexus was intensely positive with staining limited to the luminal and basal surfaces. The epithelium of the fallopian tube, uterus, and epididymis was highly immunoreactive. The acinar cells of the breast, submandibular salivary, and bronchial glands also showed intense staining as did the trophoblastic cells of the placenta. In the kidney reactivity was localized to the proximal tubules. Lung alveolar lining including type I and II pneumocytes stained intensely. Limited but focal reactivity was noted in the vas deferens, ovary, thyroid, and pancreas. This study in conjunction with previous work showing marked overexpression of folate receptor in some malignant cells suggests that the folate receptor may be an important target for diagnostic or therapeutic exploitation and indicates sites of potential drug toxicity.

Introduction

The folate receptor is a GPI-linked membrane glycoprotein that initiates cellular accumulation of 5-methyltetrahydrofolate acid in a number of epithelial cells in vitro (1). Recent evidence further suggests that antigens such as methotrexate, 5,10-dideazatetrahydrofolate acid, CB-3717, and ICI-198,583 are substrates for the folate receptor (Ref. 2; reviewed in Ref. 3). During the last 15 years several brief reports of elevated serum folate binding capacity with certain malignancies (4, 5), in pregnancy, and in some patients with severe renal or hepatic disease (6) have suggested that receptor regulation or concentrations are altered in selected disease states. Recently, Colnaghi et al., (7, 8) identified a GPI anchored, Mr 38,000 glycoprotein (gp38) that was overexpressed in human ovarian carcinoma and recognized by two monoclonal antibodies, MOv18 and MOv19. Purification of the protein and cloning of the complementary DNA identified gp38 as the folate receptor (9, 10). We and others have shown relative overexpression of gp38 in several malignant cell lines (11) and biopsy material from ovarian and renal cell carcinomas and primary brain tumors (7, 8, 11–13). In addition, the receptor has been mapped to 11q13 (9), a region amplified in greater than 20% of samples from breast and head and neck tumors (14, 15). This information has resulted in the development of potentially selective immunotherapy that has been used successfully against human ovarian carcinoma in a murine xenograft model (16). However, knowledge of folate receptor distribution in normal tissue is critical to understanding the response and potential toxicity of this form of therapy. This information may also provide additional insight into receptor mediated folate uptake via a novel process termed potocytosis (1).

Materials and Methods

Reagents and Supplies. Silane coated slides (Catalogue No. ss-770) were obtained from Histology Control Systems (Glen Head, NY). Cryotemp Mount (Catalogue No. M02) was purchased from Biomedia Corporation (Foster City, CA). Cytocool cryospray (Catalogue No. 8321) was obtained from Stephens Scientific (Riverdale, NJ). OCT compound (Catalogue No. 4583) was obtained from Miles, Inc. (Elkhart, IN). Acetone, xylene, isopentane, and Accumount mounting medium were purchased from Baxter Healthcare Corporation (McGaw Park, IL). All other salts, buffers and stains were obtained from Sigma Chemical Company (St. Louis, MO).

Antibodies and Blockers. Mouse monoclonal antibody (MOv19) against gp38 has been described by Colnaghi et al. (7, 8). Immunohistology assay (goat anti-mouse IgG-streptavidin conjugate and color development reagents) kit (Catalogue No. k0680) was purchased from Dako Corporation (Carpinteria, CA). An avidin and biotin blocking kit (Catalogue No. HK102–5k) and mouse (background control) nonimmune serum (Catalogue No. HK119–05M) was obtained from Biogenex Laboratories (San Ramon, CA).

Tissue Preparation and Immunohistology. Tissues were obtained 10–14 h postmortem and snap frozen in OCT compound by immersion in isopentane cooled by liquid nitrogen or by freezing in a cryostat with either a heat sink or cryospray. The staining procedure was modified from that used by Veggeland et al. (13). Frozen sections (5 μm thick) were placed upon silane or poly-lysine coated glass slides and air dried for 1–2 h. Immediately prior to use slides were acetone fixed at 4°C for 10 min and air dried. They were washed in phosphate buffered saline (pH 7.4) for 15 min and endogenous peroxidase activity was quenched by a 15-min immersion in 3% H2O2. After rinsing with PBS tissues were incubated with nonimmune goat serum for 5 min, rinsed with PBS, and blocked for avidin and biotin for 15 min with intervening PBS rinses. The slides were then incubated for 15 min in Hanks’ balanced salt solution containing 0.03% bovine serum albumin. Mouse monoclonal MOv19 antibody was diluted to a concentration of 0.2 μg/ml (0.04 μg/ml for choroid plexus) and applied to the slides for 30 min at room temperature. Secondary goat anti-mouse antibody application and color development using AEC chromogen were performed according to the manufacturer’s instructions. The slides were counterstained with Mayer’s hematoxylin and mounted in Crystal Mount. Matching control slides were run under identical conditions except that nonimmune mouse serum was substituted for the MOv19 antibody. Choroid plexus was run as a positive control. Blocking studies were performed utilizing MOv19 antibody that had been incubated for 30 min at room temperature.

Received 8/14/92; accepted 10/12/92.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by NIH Grant 1RO1-CA52625 and ACS CH-228F.

S. D. W. was supported by National Cancer Institute Fellowship Training Grant 5T32-CA09640–02. B. A. K. is a Burroughs-Wellcome Scholar in Pharmacology.

2 To whom requests for reprints should be addressed, at Department of Pediatrics, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235.

3 The abbreviations used are: GPI, glycosyl-phosphatidylinositol; PBS, phosphate buffered saline.
temperature with purified human placental folate receptor. Aliquots of MOv19 either undiluted (2 mg/ml) or diluted to a final concentration of 0.2 μg/ml in PBS were incubated with equal volumes of folate receptor at either 5 or 0.05 μg/ml.

Results

Immunohistochemical Controls. Preincubation of the primary antibody (MOv19) with purified human placental folate receptor eliminated immunohistochemical staining in previously positive tissues. Nonspecific binding of the monoclonal antibody was minimal since replacement of MOv19 with nonimmune mouse serum stained only limited foci in the lung (alveolar macrophages), testis (Leydig cells), breast (luminal secretions), placenta (stromal macrophages), and intestines (Paneth cells). Liver, spleen, myocardium, myometrium, adrenal, cerebrum, cerebellum, spinal cord, thymus, testis, and small intestine failed to show any specific immunohistochemical reactivity for the folate receptor.

Choroid Plexus. Choroid plexus staining was intense but limited to the epithelium and was eliminated when MOv19 was preincubated with human folate placental binding protein. Immunoreactivity persisted even when the primary antibody (MOv19) was further diluted 1:50,000 to a final concentration of 0.5 ng/ml. In some preparations a distinct bilaminar pattern was observed (Fig. 1A). An intense reaction along the luminal border was mirrored by a weaker reaction along the basal aspect of the epithelium.

Breast. Acinar cells showed reactivity to MOv19 as did the ductal epithelium. There was strong staining of luminal contents but this was also observed with nonimmune serum.

Salivary Gland. Immunoreactivity of the submandibular salivary gland was prominent in the serous acinar cells (Fig. 1B). In some acini a bilaminar pattern was observed. The intercalated, striated and transport ducts also stained at least moderately and with a more distinct bilaminar pattern. Staining of the mucous acinar cells or connective tissue septa was not observed.

Kidney. There was intense immunoreactivity of the proximal tubules (Fig. 1C). Identification of the proximal tubules was verified with Tetragonolobus purpureas (lotus) lectin staining (data not shown). An occasional collecting duct also reacted and in some preparations there was staining of luminal casts. Bilaminar staining of the proximal tubular epithelium was observed in some preparations (Fig. 1C). Distal tubules, loops of Henle, and glomeruli were nonreactive.

Ovary, Fallopian Tube, and Uterus. Ovarian staining was limited to the granulosa cells surrounding the oocytes in the cortex. This was most pronounced in the prepubertal organ.

---

Fig. 1. Immunohistochemical localization of the folate receptor. A, choroid plexus with MOv19. (~250). B, submandibular salivary gland. (~100). C, kidney. (~400). D, prepubertal ovary. (~400). E, endometrium. (~250). F, lung. (~400). G, vas deferens. (~250). Localization of the folate receptor was done with MOv19 and immunoperoxidase. All tissues were counterstained with Mayer's hematoxylin.
due to the abundance of primary follicles (Fig. 1D). In some preparations this reaction was limited to the cell margin bordering the oocyte. No staining was detected in the stromal tissue or the surface epithelium. A corpus luteum of pregnancy and the endometrium (Fig. 1E). The endometrial stroma was nonreactive.

Placenta. Intense staining was observed in syncytiotrophoblastic cells of the mature placenta. A strong reaction was also observed in the stromal cells of the villi, but this persisted undiminished in the nonimmune control.

Lung. A strong reaction was observed in the alveolar lining cells of the lung (Fig. 1F). This included both type I and type II pneumocytes. The bronchiolar and bronchial epithelium also was intensely reactive as were the acini of bronchial glands. Background staining with nonimmune mouse serum was limited to alveolar macrophages. This likely represents nonspecific binding of the mouse antibody to $F_c$ receptors on the alveolar macrophage cells.

Testis, Epididymis, and Vas Deferens. Staining in the testis was limited to the Leydig cells but these cells also reacted with the control nonimmune serum. There was staining of the epididymal epithelium, which was most intense in the body before the tubules acquired a smooth muscle coat. Staining in the head and tail was less intense and in the latter was limited to the basal epithelial cells. The basal cells of the vas deferens epithelium reacted strongly, whereas the more superficial epithelial cells were nonreactive (Fig. 1G).

Pancreas and Thyroid. These tissues had limited but focally intense reactions by immunohistochemistry. Thyroid tissue obtained from an infant showed staining of cuboidal epithelial cells in some but not all follicles. In adults, most follicular cells were nonreactive; however, the epithelial cells were reactive in an occasional small follicle. There was no parafollicular, stromal, or colloid immunoreactivity. Immunoreactivity in the pancreas was limited to the ducal epithelium. Neither the islet cells nor the acini showed any staining.

Discussion

Accumulation of 5-methyltetrahydrofolate by some cells is dependent on a GPI anchored receptor in a process called potocytosis (1). This receptor mediated process increases the efficiency of folate uptake 30-50-fold when accumulation is assessed at physiological folate concentrations (2 nm) (17). This receptor may also mediate the uptake of folate analogues that are inhibitors of purine or pyrimidine biosynthesis. The role of the receptor in methotrexate uptake has recently been reviewed by Antony (3). It appears that cellular overexpression of the receptor on cells grown in physiological concentrations of 5-methyltetrahydrofolate can result in enhanced uptake and cytotoxicity of this antifol (18). It has also been shown that certain inhibitors of thymidylate synthase (CB-3717, ICI-198,583) bind to the receptor with affinity equal to that of 5-methyltetrahydrofolate (2). Finally, 5,10-dideazatetrahydrofoleric acid, which inhibits 5'-phosphoribosylaminomide formyltransferase, also enters the cell utilizing the folate receptor (3). Thus the presence of machinery for receptor coupled transport not only confers a growth advantage to cells exposed to limited 5-methyltetrahydrofolate concentrations but may also be exploited as a highly efficient delivery system for antifols.

We recently reported the limited expression of the folate receptor in a large number of normal human tissues and the overexpression of the receptor in certain primary brain tumors by immunoblotting with MOv19, radioimmunoassay with MOv18 and MOv19, and Northern blot analysis (11, 12). In a survey of nearly 20 tissues, the choroid plexus consistently expressed the largest amount of receptor. Other tissues expressing detectable amounts of folate receptor by immunological technique included lung, kidney, and thyroid. The pancreas was minimally reactive. In an immunohistological survey limited to normal genitourinary tissues, Vegglan et al. (13) observed immunoreactivity in the oviduct epithelium and renal tubules (both proximal and distal). They found no reactivity in the vas deferens, epididymis, endometrium, or ovarian follicles; however, they used MOv18 rather than MOv19 in their study. No other immunohistochemical studies have been reported.

In the present study, all tissues previously positive for receptor by immunoblotting of membrane preparations (11) showed at least focal immunohistochemical staining. Similarly, all tissues that were negative for receptor by immunoblot failed to show reactivity by immunohistochemical analysis. The presence of the folate receptor in the salivary gland, vas deferens, epididymis, and mammary gland has not been described previously, although there have been reports of folate binding protein in saliva, seminal fluid, and milk (19, 20). Historically, folate receptor was first isolated from milk (21) and its presence in breast acini and ducts provides a correlate for this observation.

Any explanation for the function(s) of receptor in these various sites must account for its distinctive distribution. The apparent bilaminar localization of the receptor in the choroid plexus and the proximal renal tubules suggests a transport or storage role in these tissues. Unlike previous studies with cell lines, which showed that folate is concentrated within cells (1, 11, 17, 18), the present study suggests that the choroid plexus may serve to concentrate or conserve this vitamin within the spinal fluid where a spinal fluid:serum ratio of 3:1 exists (22). Localization of renal immunoreactivity to the proximal tubules supports the hypothesis that the receptor functions in the kidney to conserve folates (23). The intraluminal immunoreactivity observed within the renal tubules most likely represents solubilized receptor (folate binding protein) present in the urine as reported previously (19). Receptor in ductal epithelium (e.g., pancreas, vas deferens, and salivary gland) may also serve as a means to conserve folates. Such an extensive conservatory mechanism could explain, in part, the low dietary requirement for folate and the difficulty with which stores are depleted.

The cellular distribution of the folate receptor may provide insight into the observed toxicity of inhibitors of purine and pyrimidine biosynthesis and suggest potential sites of concern. Both the lung (bronchiolar and bronchial epithelium, pneumocytes) and kidney (proximal tubules) have significant concentrations of receptor and are known targets of methotrexate toxicity (24). Renal proximal tubular necrosis (25) and pulmonary fibrosis or interstitial pneumonitis (26) can occur following methotrexate exposure. Mucositis, which frequently occurs with methotrexate administration, appears to correlate with the concentration of this antifol in saliva and duration of mucosal exposure (27). Whether receptor mediated uptake by the salivary glands contributes to the drug concentration in saliva and duration of mucosal exposure remains to be determined. The localization of receptor in the ovarian granulosa cells is distinctive and might contribute to the defective oogenesis seen
with this agent. Since methotrexate toxicity also occurs in receptor negative tissues, different modes of cellular uptake (3) or mechanisms of toxicity may be involved. In this regard, central nervous system toxicity may result indirectly from methotrexate interference with choroid plexus function. This mechanism might contribute to the folate deficient state of brain tissue following methotrexate exposure (28).

The cellular localization of the folate receptor may be predictive of additional toxicity as newer agents such as 5,10-dideazatetrahydrofolic acid, CB-3717, or ICI-198,583 are brought into use. These potent agents have a 25–50-fold greater affinity for the folate receptor than methotrexate (2, 3). At this time too few clinical studies have been performed with these newer agents to correlate our observations of cellular localization of the receptor with expression of drug toxicity.

Acknowledgments

We wish to thank William Webb, H.T., A.S.C.P., and Angelia Jones, H.T., A.S.C.P., for their technical assistance. In addition, we wish to thank Dr. Tibor Nadasdy (University of Oklahoma Health Sciences Center, Department of Pathology) for performing lectin staining of renal tissue.

References

Cellular Localization of the Folate Receptor: Potential Role in Drug Toxicity and Folate Homeostasis


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/52/23/6708