Lectin affinity electrophoresis in a yolk sac tumour in the vagina with yolk sac tumour-type glycoform of α fetoprotein

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Abstract

Aims—To investigate a potential diagnostic use of α fetoprotein (α FP) isoform analysis by lectin affinity electrophoresis to distinguish between endodermal sinus tumours arising in the vagina in infants from those at other sites.

Methods— α FP in the serum of a patient with a vaginal endodermal sinus tumour was analysed for its isoforms by lectin affinity electrophoresis. The isoforms were compared with that of cord serum, sera of hepatoid adenocarcinoma of the uterus, and endodermal sinus tumour of the ovary.

Results—The isoforms of α FP obtained by lectin affinity electrophoresis in the serum of the patient with vaginal endodermal sinus tumour differed from the isoforms of α FP in the cord serum of normal neonates, and sera of patients with hepatoid adenocarcinoma of the uterus or endodermal sinus tumour of the ovary.

Conclusions—Endodermal sinus tumour arising in the vagina could be distinguished from that in the ovary by the lectin affinity electrophoresis, and a potential diagnostic use of α FP isoform analysis by the lectin affinity electrophoresis for the detection of the endodermal sinus tumour in infants was demonstrated.

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Human serum α fetoprotein (α FP) having one asparagine linked sugar chain is one of the most reliable tumour marker of malignancies, particularly hepatocellular carcinomas and endodermal sinus tumours.¹ Sugar chain heterogeneity of human α FP was first demonstrated by Smith and Kelleher² by means of affinity chromatography with concanavalin A (conA) bound to agarose gel. Breborowicz *et al*³ and Miyazaki *et al*⁴ separated α FP variants of patients with hepatocellular carcinomas by crossed immuno-affino-electrophoresis and demonstrated a potential diagnostic use, but the sensitivity of this method for detection of separated α FPs was limited. Taketa *et al*⁵ introduced antibody affinity blotting followed by immunoenzymatic amplification, which produced not only an increase in the sensitivity of detecting separated α FP bands but also enabled

a direct comparison of band mobilities by parallel running of samples. α FPs derived from hepatocellular carcinoma, germ cell tumours of the ovary, and other α FP producing carcinomas were separated into several isoforms corresponding to the difference in sugar chain structures by lectin affinity electrophoresis.^{6,7} This is the first report to demonstrate that endodermal sinus tumours arising in the vagina in infants can be distinguished clinically from those in ovaries by α FP isoform analysis using lectin affinity electrophoresis.

Methods

PATIENT

A 12 month old Japanese patient was admitted to our hospital with abnormal vaginal bleeding of a months duration. Routine blood and urine studies were normal except for raised α FP (25.0 μ g/ml; normal <10 ng/ml). Pelvic computed tomography (CT) showed a non-specific soft tissue mass located between the urinary bladder and rectum with a left inguinal lymph node swelling. Pelvic magnetic resonance imaging (MRI) showed a prominent irregular mass in the vagina. A vaginal Papanicolaou smear with a cotton-tip applicator showed no abnormal cells. Biopsies of the tumour were performed under general anaesthesia through the vagina and the histological diagnosis was endodermal sinus tumour (fig 1).

Six courses of combination chemotherapy were instituted using intravenous bleomycin hydrochloride (0.5 mg/kg/day) on day 2, etoposide (4 mg/kg/day) on days 1, 2, and 3, and cisplatin (0.13 mg/kg/day) for five consecutive days every 3-8 weeks. The patient underwent a total abdominal hysterectomy, bilateral salpingectomy, and pelvic lymph node dissection with total removal of the vagina. At surgery the corpus and cervix of the uterus, bilateral fallopian tubes, and ovaries were grossly intact. A solid mass (2.2 $\times$ 1.8 $\times$ 0.7 cm) was found in the upper third of the anterior to right lateral vaginal wall. Microscopic examination revealed the remaining viable endodermal sinus tumour cells arising from the vagina, which showed alveolar-glandular pattern. Immunohistochemical stain demonstrated the presence of α FP in the tumour cells (fig 2).

LECTIN AFFINITY ELECTROPHORESIS

α FP in the serum of the patient was analysed for isoforms by lectin affinity electrophoresis as described previously.⁵ Briefly 4 μ l of serum

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Figure 1 Microscopic findings of the biopsied tissue. Yolk sac vesicles–vitelline pattern is illustrated (haematoxylin and eosin; original magnification $\times 200$).

Figure 2 Immunohistochemical staining for α FP in a surgical specimen. The sections of the tumour, fixed in formalin and embedded in paraffin wax, were stained by the unlabelled antibody peroxidase–antiperoxidase method using rabbit antihuman α FP sera. The tumour cells showed positive staining for α FP in the cell cytoplasm (original magnification $\times 200$).

Figure 3 Lectin affinity electrophoresis of α FPs. α FPs in the sera of the umbilical cord of a normal neonate (cord serum), a patient with hepatoid adenocarcinoma of the uterus, a patient with endodermal sinus tumour (EST) of the ovary, and a patient with EST of the vagina were electrophoresed in agarose gels containing concanavalin A (ConA), *Lens culinaris* agglutinin A (LCA), erythroagglutinating phytohaemagglutinin of *Phaseolus vulgaris* (E-PHA), and *Allomyrina dichotoma* lectin (alloA).

samples diluted to contain 100 ng/ml of α FP were applied to an agarose gel plate containing conA, *Lens culinaris* agglutinin A (LCA), erythroagglutinating phytohaemagglutinin of *Phaseolus vulgaris* (E-PHA), and *Allomyrina dichotoma* lectin (alloA). Following electro-

phoresis, the α FP was transferred to a nitrocellulose membrane pre-coated with monoclonal mouse antibody to human α FP. The membrane was reacted with $F(ab')_2$ fragments of rabbit immunoglobulins to α FP and then with affinity purified goat antirabbit IgG conjugated with horseradish peroxidase. α FP bands were visualised by detecting the enzyme activity by the tetrazolium method. The major α FP bands were numbered consecutively from the anode and the band numbers were suffixed to the capitalised initial letters of lectins used. Slow and fast migrating bands of α FP-P3 was shown as α FP-P3s and α FP-Pf, respectively. Samples analysed in parallel experiments were from our frozen serum stocks and included cord serum from normal neonates, sera from a patient with hepatoid adenocarcinoma of the uterus,⁹ and a patient with endodermal sinus tumour of the ovary.

Results

α FP isoforms corresponding to the different sugar chain structures were demonstrated by lectin affinity electrophoresis. With conA, α FP can be separated into two fractions, conA non-reactive (C1) and conA reactive (C2). As shown in fig 3, the two α FP isoforms were clearly separated in the serum of uterine hepatoid, ovarian endodermal sinus tumour, and vaginal endodermal sinus tumour, while the cord serum showed minimal C1 isoform. LCA separated α FP into three fractions, L1, L2, and L3. The α FP from vaginal endodermal sinus tumour displayed L2 and L3. The isoform profiles of α FP from four different origins were distinct from each other. Four major fractions, P2, P3, P4, and P5 and one subpopulation, P3f, were demonstrated with E-PHA. The α FP from vaginal endodermal sinus tumour displayed P2, P3f, P4, and P5. This isoform profile was distinct from those of ovarian endodermal sinus tumour. With alloA two major fractions, A1 and A3, and one subpopulation, A1s, were demonstrated. The α FP from vaginal endodermal sinus tumour displayed A3 and a broad fused band of A1 and A1s. This pattern was distinct from those of other sources.

Discussion

Endodermal sinus tumour is a malignant germ cell neoplasm generally originating in the ovaries and testes of young patients¹⁰ between the ages of 10 and 30 years. It has been encountered in extragonadal sites including the vagina,^{11–13} endometrium,¹⁴ and vulva.¹⁵ Endodermal sinus tumour arising in the infant vagina is extremely rare. Approximately 50 cases of vaginal endodermal sinus tumour have been reported in the literature and all of them presented before 3 years of age. Endodermal sinus tumour of the vagina has histological characteristics similar to endodermal sinus tumour of the ovary. Both contain yolk sac vesicles–vitelline pattern, and α FP in the tumour cells can be demonstrated by immunohistochemical methods. Because of the histological similarities, a distinct pathological diagnosis has not been established. Clinically,

pelvic CT and MRI are used for distinguishing both types of tumours. In the present case the origin of the tumour was indistinguishable by pelvic CT; MRI showed the location of the primary tumour in the vagina. The current treatment for endodermal sinus tumour of the vagina is preoperative combined chemotherapy, followed by surgical resection preserving the bladder and rectum. Radiation can be given on the basis of the microscopic findings of surgical specimens.

While both CT scan and MRI provide useful information on which to base treatment, the use of α FP isoforms provides a new non-invasive diagnostic method by which endodermal sinus tumours at different sites can be distinguished. The technique has the advantage of avoiding the need for anaesthesia that may be necessary to ensure adequate imaging of uncooperative neonates, and avoids the need for ionising radiation. It was demonstrated that endodermal sinus tumour elements are associated with α FP synthesis, and the α FP synthesised by tumour cells has one asparagine linked sugar chain.¹ Human serum α FP is one of the most reliable tumour markers of endodermal sinus tumours originating in the ovary and extragonadal sites that can be used to monitor the effectiveness of treatment and to detect recurrences. α FPs derived from germ cell tumours composed entirely of or containing endodermal sinus tumour elements were separated into several isoforms by the lectin affinity electrophoresis corresponding to the different sugar chain structures, α FPs with high affinities for a lectin being retarded and those without affinity for the lectin not being retarded.¹⁶

Clinical applications of lectin affinity electrophoresis for early detection of hepatocellular carcinoma,¹⁷ distinction of hepatocellular carcinoma from endodermal sinus tumour and metastatic liver cancer¹⁸ have been reported. Yamashita *et al*¹⁹ suggested that the isoform specific to hepatocellular carcinoma is a useful tumour marker to differentiate it from benign liver diseases, including liver cirrhosis and chronic hepatitis, based on the hypothesis that fucosylation of α FP may be related to the differentiation of human hepatocytes through tumorigenesis. In the present paper we demonstrated that tumours having similar histological characteristics and originating from different organs could be distinguished by sugar chain heterogeneity of α FP. The results of α FP isoform analysis suggested that a set of

glycosyltransferases that was different from other types of cells was operating in the vaginal endodermal sinus tumour cells to generate the unique carbohydrate structure. This is the first report to demonstrate the potential diagnostic use of α FP isoform analysis by the lectin affinity electrophoresis for the detection of the endodermal sinus tumour in infants.

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