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Developmental Changes in Expression of a Tumor-Associated Protein in the Rat Fetus

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Monoclonal antibodies specific for a rat tumor-associated protein cross-react with a similar protein present in the cytosol of the rat fetus. The oncofetal protein exists as two species of approximate molecular weight 50 and 55 kDa which promote the transport of RNA from isolated nuclei. During rat fetal development, the protein first increases in concentration from approximately 12 to 16 days gestation and then drops to non-detectable levels perinatally.

Key words: oncofetal protein, monoclonal antibodies, RNA transport, fetal development, cytosol

A novel protein (OFP) was identified in the plasma of tumor-bearing rats by virtue of its ability to induce RNA transport from isolated nuclei in a cell-free system [1]. This protein is produced by all tumors [2] but not by normal (non-neoplastic) tissues in experimental animals or human subjects [3]. OFP is induced in target tissue during chemical carcinogenesis in the rodent system and its production persists in the tumors produced [4,5]. In the present study, a series of monoclonal antibodies produced against OFP released from the rat hepatoma 7777 are used to evaluate changes in the oncofetal protein during fetal development.

Sprague Dawley–timed, first-time-pregnant rats (Harlan Labs, Indianapolis, IN) of approximately 250 gm body weight were maintained on a standard chow diet. Embryonic tissue was homogenized in 50 mM Tris-HCl, 5.0 mM $MgCl_2$, 25 mM KCl, pH 7.5 (TMK buffer) 0.25 M sucrose, 10 mM phenyl methyl sulfonyl fluoride and then centrifuged at 105,000*g* for 90 min to produce the cytosol. Balb/c mice were immunized with 1.0 mg/ml of the purified protein [2] in saline which was emulsified with Freund's adjuvant. Hybridomas [6] were obtained by fusing spleen cells with the Balb/c myeloma cell line P3 × 63/Ag 8.653 in the laboratory of Dr. Bruce Zwilling, Dept. of Microbiology, Ohio State University. The antibodies were purified from the hybridoma culture medium by use of Sepharose-immobilized protein G (Pharmacia, Piscataway, NJ). For the immuno-bioassay, monoclonal antibodies were immobilized by forming an immune complex with agarose bead-linked anti-mouse IgG (H + L; Sigma Chem. Co., St. Louis, MO) by incubation for 18 h at 4°C in 0.01 M phosphate buffer, pH 7.2–0.25 M

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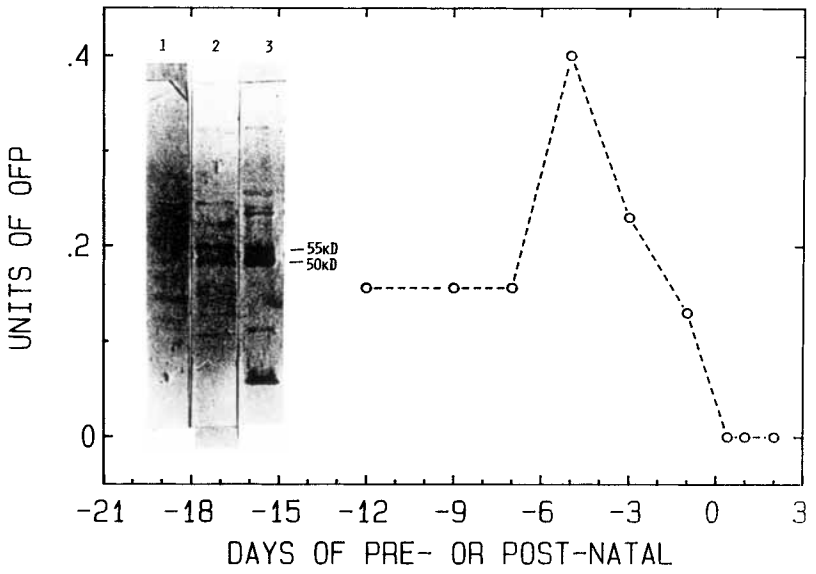

Fig. 1. Temporal changes in the putative 60 kDa oncofetal protein during fetal development as assessed with the monoclonal antibody-based immunobioassay. Birth is at day 0. **Inset:** Western blots of the oncofetal protein isolated from the cytosol of the rat fetus and of the hepatoma 7777. **1:** Normal liver cytosol. **2:** Fetal cytosol. **3:** Tumor cytosol.

NaCl. Non-specific binding sites were blocked by incubation with 2% dry milk in 0.5 M NaCl–TMK buffer [7]. After washing, the beads were resuspended in the same buffer and 20 μl was incubated with the test sample containing the factor. The agarose beads were removed by centrifugation and an aliquot was tested in the biochemical assay described previously [1–5]. Oncofetal protein is estimated from the difference in RNA transport activity before and after treatment with the beads. One unit is the % nuclear counts released in 30 min at 30°C. Immunoblots were performed as described [7].

Shown in Figure 1 is the temporal change in the concentration of the oncofetal (oncodevelopmental) protein during rat fetal development as determined by the monoclonal antibody-based immuno-bioassay. OFP remains at a constant level from 9 to 14 days gestation and then increases to peak at 4 units at 16 days (5 days prenatal). It then decreases sharply and reaches non-detectable levels around day 20, i.e., just before birth. OFP remains at non-detectable levels post-natally, except for its reappearance in the tumor if carcinogenesis intervenes [4,5]. Insufficient material was available for evaluation prior to 12 days. The monoclonal antibodies did not detect any of the putative oncofetal protein in the maternal plasma of the 16-day pregnant rat although it is present in the blood from tumor-bearing rats. This indicates that OFP does not cross the placental barrier to maternal circulation (D.E. Schumm and T.E. Webb, unpublished data).

Shown in the insert of Figure 1 are Western (immuno-) blots of the oncofetal protein present in the cytosol of the 16-day rat fetus and of the rat hepatoma 7777. The two species in the cytosol of the tumor and fetus appear identical in molecular weight. The molecular weights of the two species are estimated to be 50 and 55 kDa. These factors are not detectable in normal liver cytosol.

The oncofetal protein described in this communication is an important marker protein since it is present in all tumors and is induced in the target tissue early in carcinogenesis. The results of the present investigation indicate that this protein is maximally induced during the final third phase of fetal development.

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