BIKEN JOURNAL Vol. 20 143-145, 1977

SHORT COMMUNICATION

EFFECT OF OOPHORECTOMY ON ESTROGEN RECEPTORS IN RAT MAMMARY TUMORS INDUCED BY 7,12-DIMETHYLBENZ(A)ANTHRACENE

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(Received April 25, 1977)

Extensive studies on estrogen receptors have been reported by many workers and it has been shown that the presence of the cytoplasmic estrogen binding protein in mammary cancer tissue is closely related with estrogen dependency of the tumors (McGuire et al., 1975). Recent studies revealed that the growth of mammary cancer tissue was also dependent on progesterone (McGuire, 1976), prolactin (Meites, 1969; Pearson et al., 1969, Vignon and Rochefort, 1976) or other hormones (Cohen, 1974). Surgical ablations, such as oophorectomy, adrenalectomy and hypophysectomy, were effective in more than half the patients with breast cancer in whom estrogen receptors were found in tumor tissues, although not all tumors with estrogen receptors responded to these surgical ablations. In the present work, we examined the relation between the response of tumors to oophorectomy and presence of estrogen receptors in the tumor tissues in rat mammary carcinomas induced by 7,12-dimethylbenz(a)anthracene (DMBA).

Female Sprague-Dawley rats of 55 to 60 days old, weighing 180 to 210 g, from the Breeding Station for Laboratory Animals, Osaka University, Osaka, Japan were used. They were fed on commercial food (Oriental Yeast Co., Suita, Osaka) and given water ad libitum. Intravenous administration of DMBA (15% oil emulsion, 25 mg per Kg body weight, kindly provided by Upjohn Co., Kalamazoo, Michigan, USA) induced mammary tumors in all the rats, as reported previously (Takeda et al., 1975). Animals with solid tumors (1.5 to 2.0 cm in diameter) were used for experiments.

Small parts of the tumors were removed under sterile conditions and immediately tested for the presence of estrogen receptors. Hemostasis was performed carefully to the site of operation and then interrupted silk sutures were applied. Two diameters of the residual tumors were measured at right angles to each other and then bilateral oophorectomy was performed. The size of tumors was measured every other day. The residual tumors were removed 7 to 11 days after surgery and their content of estrogen receptors was determined. In some rats, tumors were partially resected and later estrogen receptors were determined again, the size of the residual tumor

being measured every other day for about two more weeks.

Estrogen receptors were measured as follows: Tumors (0.45 g wet weight) were homogenized in 1 ml of ice cold 0.01 M Tris (hydroxymethyl) aminomethane-HCl buffer (pH 7.4) containing 0.0015 M EDTA and 0.002 M 2-mercaptoethanol in a glass homogenizer with a teflon pestle. The homogenate was centrifuged at 105,000×g for 1 hr and the supernatant was mixed with 5 μliters (0.5 μCi) of a solution of ^{3}H-estradiol-17β (specific activity 49.3 Ci per mM) in ethanol. The mixture was incubated with shaking at 0–2 C for 30 min, layered on 4.8 ml of a 5 to 20% sucrose density gradient, and then centrifuged at 114,000×g for 16 hr at 2–4 C. Then 0.25 ml fractions were collected from the bottom of the tube and their radioactivities were determined in the scintillation medium described previously (Tominaga et al., 1975).

Estrogen receptors were found in 18 of 33 tumors examined (54.5%). No special correlation was found between the histological differentiation of the mammary carcinomas and the presence of estrogen receptors, and there was no relation between the time of tumor induction after DMBA administration and the presence of estrogen receptors.

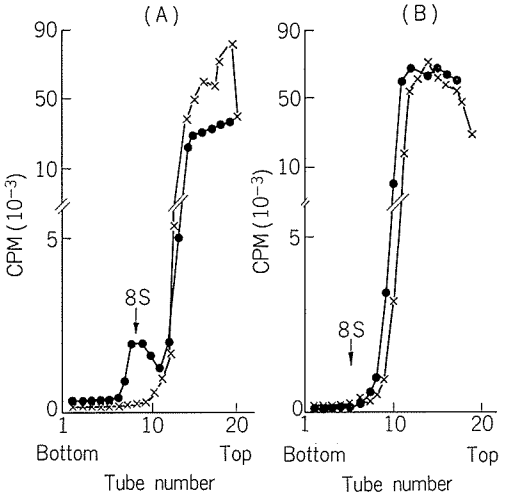

FIGURE 1 Effect of oophorectomy on estrogen receptors in tumors. Estrogen receptors sediment at 8S. Tumor before castration (●·····●). Tumor after castration (×·····×).

When oophorectomy was performed in 18 rats in which estrogen receptors had been demonstrated, the receptors disappeared several days after surgery in 17 of the rats, but in the 18th rat estrogen receptors were still present 7 days after oophorectomy. All but one of the tumors with estrogen receptors decreased in size 7–11 days after oophorectomy.

Oophorectomy had various effects on the growth of 15 tumors in which estrogen receptors were not detected; 4 grew, 9 regressed and 2 remained unchanged in size. No estrogen receptors were found in any tumors after castration.

The effect of oophorectomy on the growth of tumors was investigated further. In one tumor that decreased greatly in size after oophorectomy estrogen receptors disappeared completely within 10 days after oophorectomy as shown in Fig. 1, panel A. In contrast, another tumor that had no estrogen receptors increased slightly in size after oophorectomy as shown in Fig. 1, Panel B. Thus it is concluded that growth of tumors is not always dependent on ovarian hormones alone.

The time courses of changes in size of tumors after oophorectomy and administration of estradiol-17β are shown in Fig. 2. In rat A, in which estrogen receptors were demonstrated, the tumor continued growing after a sham operation. In rat B, which no estrogen receptors were demonstrated, the tumor also increased in size after a sham operation. No estradiol-17β was administered to either rat A or rat B. In rat C, no estrogen receptors were demonstrated and the tumor did not change in size after castration. No estrogen receptors were found 8 days after the operation, but the tumor began to grow again after administration of estradiol-17β. In rat D, no estrogen receptors were demonstrated on day 0, and the tumor rapidly decreased in size after castration. On day 8, estrogen receptors were still not detectable, but the tumor began to

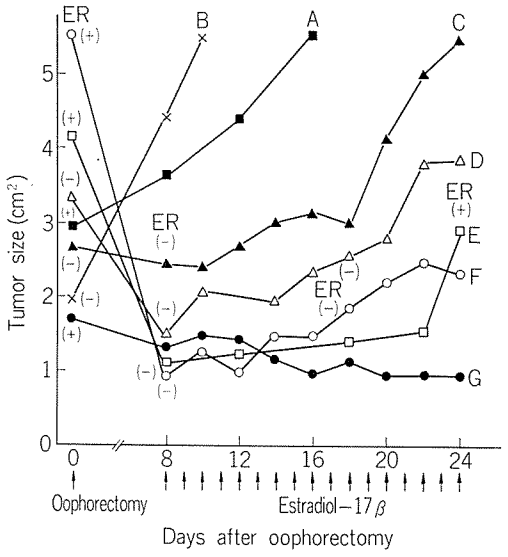

FIGURE 2 Effects of oophorectomy and administration of estradiol-17β on tumor size. Seven tumors from different rats (A-F) were examined. Castration was carried out on day 0. (+) and (−) indicate the presence and absence, respectively, of estrogen receptors. Estradiol-17β (0.4 μg/Kg body weight) was injected intramuscularly every day from day 8 to 24 after castration.

grow again on administration of estradiol-17β. Estrogen receptors did not appear by day 18 after castration. In rat E, estrogen receptors were demonstrated on day 0 and the tumor decreased rapidly in size after oophorectomy. On day 8, no estrogen receptors were found, but on daily administration of estradiol-17β the tumor started to grow and estrogen receptors had reappeared on day 24. Similarly in rat F, in which estrogen receptors were present initially the tumor decreased greatly in size and estrogen receptors had disappeared by day 8 after the operation. The tumor started to grow on administration of estradiol-17β, although no estrogen receptors were found on day 18 after castration. In rat G, in which estrogen receptors were demonstrated, the tumor did not change in size after oophorectomy but estrogen receptors disappeared by day 8 after the operation, and no tumor growth was observed after administration of estradiol-17β. These results suggest that tumor growth is closely related with estradiol-17β, but not with the presence of estrogen receptors. As suggested by the work of McGuire (1976), Vignon and Rochefort (1976) and Cohen (1974) it is likely that hormones other than estrogen such as progesterone and prolactin are important in growth of mammary carcinomas. A preliminary report of this work has been presented elsewhere (Tominaga, 1976).

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