HISTOLOGIC ORIGIN OF RAT OVARIAN CANCER INDUCED BY DIRECT APPLICATION OF 7,12-DIMETHYLBENZ(A)ANTHRACENE

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Synopsis A direct application of 7,12-bis(dimethylbenz(a)anthracene (DMBA) to the ovary successfully produces an ovarian epithelial cancer in rat. To identify the histogenic process, the DMBA-treated rats were serially examined from the 15th to the 40th week following DMBA application. Furthermore, from the anticipated effect of estrogen on surface epithelial proliferation, the rats were put into an hyperestrogenic condition for immunohistochemical characterization of the tumor tissue. At the 20th week, the surface epithelial proliferations were seen to show remarkable multistratification with cellular polymorphism. The proliferated surface cells began to infiltrate into the stroma to form irregular gland structures. In immunoperoxidase stainings, both the adenocarcinoma cell and the surface cell has the same character in the immunohistochemical reaction for estradiol. On the base of these results, the induced cancer in rat was believed to have a similar histogenic process to the common epithelial tumors in human ovary.

Key words: Rat ovarian cancer • 7,12-dimethylbenz(a) anthracene • Histologic origin • Immunohistochemistry

Introduction

A rat ovarian cancer induced by local application of 7,12-dimethylbenz(a)anthracene (DMBA) is an interesting model for experimental therapeutic study because of its similarity in biologic behavior and histologic feature to the epithelial malignancy in human ovary, showing: 1) the primary tumor development in the ovary, 2) intraperitoneal dissemination occasionally with bloody ascites, 3) histology of adenocarcinoma and finally 4) presence of steroid hormone receptors in the tumor tissue.

To evaluate experimental models for human tumor, in the general, it is important to identify the histologic origin. The common epithelial tumors in human ovary are accepted as derivatives from the ovarian surface cells, while the origin of the DMBA-induced cancer in rat, as described by Murphy in his evaluation of various experimental models in 1980, has not yet been confirmed.

To discern the histogenic process of the induced cancer, the morphologic changes of rat ovary were serially observed from the fifth to the 40th week following DMBA application. Further, because of the expectable role of estrogen, as previously reported, playing on promotion of the carcinogenic process in the DMBA-treated rat ovary, the rats were set into hyperestrogenic condition for immunohistochemical characterization of the tumor tissue.

In the present paper, the histogenic process of the rat ovarian "epithelial" cancer is morphologically demonstrated, and the methodology of producing experimental model by chemicals will be shortly discussed.

Materials and Methods

Thirty female Wistar strain rats were used in this study at the age of six weeks. Under ether anesthesia, the left ovary was exposed surgically from the membranous pouch in which the ovary had lain. A DMBA (Wako Pure Chemical Industries, LTD., Osaka) impregnated silk thread, containing 2.1 μg of the carcinogen in average, was inserted into the naked ovary and knotted. The right ovary was kept intact.

To bring hyperestrogenic environment in the rats, they received weekly administrations of 2mg/ body of an estrogen depot (Peranin Depot: Mo-
chida Pharmaceutical CO., LTD., Tokyo) from the 5th to the 20th week following application of DMBA.

From the 15th to the 19th week, two rats were examined in each week. Ten of the remainings were sacrificed immediately after the last injection of estrogen, and the other rats were killed after forty weeks from the DMBA treatment.

The tumor tissues obtained were fixed by trans-aortic perfused fixation with Bouin's solution and embedded in paraffin. For the routine histologic examinations, the tissues were stained with hematoxylin and eosin (HE) and with periodic acid-Schiff (PAS). In order to observe immunohistochemical reaction of the tumor tissue, immunoperoxidase staining for estradiol was attempted with a commercially available PAP (Peroxidase Anti-Peroxidase) kit (HISTOSET, Ortho Diagnostic Systems Inc., New Jersey).

Results

Any neoplasms originated from extraovarian tissue were not found in this series of investigations. In the ten rats examined from the 15th to the 19th week following DMBA treatment, no neoplastic changes occurred in the treated ovary.

In four of ten rats examined at the 20th week, ovarian tumor already developed, although extraovarian spreads were not observed except one case with a small metastasis on the omentum. In these tumors, focal proliferations of the ovarian surface cells, more than three layers in thickness, were noted. The proliferated cells seemed to have propensity to invaginate in the ovarian cortex. In the stroma, neoplastic cells developed to form irregularly glandular structures partly with solid cellular arrangements. The tumor cells occasionally connected with the proliferated surface epithelial cells (Fig. 1). This histologic feature and lack of PAS positive substances in the cytoplasm indicated that the induced ovarian tumors were adenocarcinomas in serous type.

Immunoperoxidase reactions for estradiol were strongly positive in these proliferated surface cells. The pleomorphic surface cells occasionally with intracellular vacuole increased in size and volume of the cytoplasm and the nuclei, and proliferated remarkably to stratify often showing gland-like lumina (Fig. 2). The similar immunohistochemical reactions were also revealed in the infiltrated cancer cells forming tubular structures (Fig. 3).

After forty weeks from the DMBA application, the induced ovarian tumor was found in six of the remaining ten rats. Three tumors showed extraovarian spreads. One of them made a quite similar picture to human ovarian cancer, showing extensive intraperitoneal developments with bloody ascites including metastases on the omentum, the mesentery and the diaphragmatic surface (Fig. 4). Free floating malignant cells were also observed in the ascites by cytological examination.

Because of the progressive feature of the tumor development, histological evidence of the surface epithelial proliferations was not able to be observed in the tumors examined at this time. However, the
intracytoplasmic antiperoxidase was also revealed by PAP technique in the neoplastic cells lining on glandular structures which infiltrated deeply in the stroma (Fig. 5).

Discussion

Opinions concerning the origin of the DMBA-induced cancer in rat ovary have been divided. On the base of histologic findings of serial sectioned tumor tissue, Iwasawa et al. found that the many of the tumors arose from epithelial proliferation in the hilar region, while we suggested that the tumor might originate from the ovarian surface epithelium throughout a series of previous investigations in our laboratory.

The results obtained in this study clearly indicate the origin of the induced cancer from two reasonable points. First, the adenocarcinoma cells developed in the stroma directly connected with the proliferated ovarian superficial cells with pleomorphism and occasionally with gland-like lumina. The second point is the common characteristic in immunohistochemical reaction for estradiol of both the adenocarcinoma cell and the surface epithelial cell. Even after twenty weeks from the last injection of estrogen, the positive immunoperoxidase reaction remained in the cancer cells.

Although definite evidence implicating steroid hormones in the development of ovarian epithelial malignancy remains to be established, a possible role of steroids playing on the surface epithelial proliferations during fetal and reproductive periods was indicated in previous ultrastructural observations on human and on animal ovaries. In addition, Katabuchi proved estrogen receptor in 80% of the DMBA cancers, and found that the cancer cells in culture were affected to proliferate by 17β-estradiol application. From these facts, we also utilized estrogen application for immunohistochemical characterization of the tumor tissue.

When considering the solubility of steroid in organic solvents, it is likely that most of steroid content of the tissue is removed following the preparation of tissue specimen. This is thought to be one of questionable points for steroid localization proved by PAP technique. In 1974, however, Bubenik et al. checked the influence of acetone
fixation on steroid content of tissue, and proved using radioimmunoassay that amount of steroid was still able to present even after prolonged fixation. In more recent investigations, Kuruman et al.\(^{8,9}\) also supported this conception and concluded that the PAP method was sufficiently sensitive to detect steroids remaining following fixation, processing and embedding. Therefore, it is reasonable to suppose that the result of PAP method in this study indicated estradiol localization in the tumor, or at least to believe that both the cancer cells and the surface cells have the same character in immunohistochemical reaction for estradiol.

The method described by Kato et al.\(^{7}\) was unique and reasonable in the experimental production of ovarian epithelial tumor. In this study, we slightly modified the original method to expose the ovary from the membraneous capsule before DMBA application, and this might help the induced cancer to develop on the peritoneal surface.

We have used DMBA in our series of experiments as a powerful carcinogenic chemical. Although much remains to solve in the ovarian epithelial carcinogenesis, there are reincreasing interests in chemical carcinogenesis in current industrialized countries\(^{10,16}\). Accordingly, with regard to the pathogenesis and the experimental procedure also, local application of DMBA to the ovary is thought to be an acceptable and reasonable method to produce animal model for ovarian tumors of the common epithelial types.

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References

概要 7,12-dimethylbenz(a)anthracene（DMBA）を卵巣に直接、局所的に作用させることで発生するラット卵巣腺癌の組織由来の究明を試みた。このDMBAによる腺癌がestrogenに感受性を有することから、DMBA処置後のラットに第5週目から20週までestrogen製剤を週1回投与し、実験開始より15週から40週後まで卵巣を検索した。20週目ですべて10例中4例に卵巣腫瘍が形成され、40週では10例中6例に転移巣を含む巨大な卵巣腫瘍を認めた。このうち1例は横隔膜下および腹膜播種と血性腹水を有していた。組織形はすべてserous typeの腺癌であり、ヒト腫瘍との共通性を示した。20週目の卵巣では一部に表層上皮の増殖が観察され、これは著明な重層化、細胞異型を示した、これら増殖した表層細胞は間質中へ陥入し、不規則な腺構造を形成する傾向をみせ、他の部分でみられる腺管構造を示す腺癌細胞と形態的に類似しており、時にはこれらは直接連結していた。さらに免疫組織化学的に増殖した表層上皮腺癌の細胞はともに細胞質中でestradiolのanti-serumに対する強い抗原性を示した。40週目における癌細胞も同様の反応を示したが、これら両細胞群の共通した性格からDMBAで誘発したラット卵巣腺癌はヒトの場合と同様に卵巣の表層上皮に由来することが示唆された。また、ヒト癌と化学物質の関係が注目されている現在、DMBAによる本法はヒト卵巣癌 modelの作製法としては理に適った実験法であると考えられた。