Expression of 3β-hydroxysteroid dehydrogenase-5,4-en isomerase activity by infiltrating ductal human breast carcinoma in vitro

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Summary

In order to determine whether human mammary tumors could contribute to progesterone synthesis from pregnenolone in breast cancer patients, homogenates of infiltrating ductal primary breast tumors at different stages of malignancy (Stages II and III) obtained from pre- and post-menopausal patients (n = 7, age 37-66 years) were incubated with [7α-{3H}]pregnenolone as substrate. Controls were heated homogenates instead of fresh homogenates. With the use of reverse-isotope dilution analysis, [3H]progesterone was isolated and characterized. No such metabolite was evident in the control incubations of heat-denatured enzymes. The extent of enzymatic conversion varied from 0.02 to 4.0%. The results reveal that activity of 3β-hydroxysteroid dehydrogenase-5,4-en isomerase that metabolizes pregnenolone to progesterone can be identified with the viable homogenates. It is suggested that there exists a potential for substantial progesterone synthesis in vivo. This conversion may be of considerable clinical, therapeutic, and pathophysiological significance in the patient with breast cancer. The biological impact of this conversion should be a high priority research objective.

Introduction

Knowledge of the sequences involved in the biosynthesis of steroid hormones allows for a rational approach to the synthesis of effective inhibitors which would have therapeutic application in the treatment of hormone-dependent metastatic breast cancer. Recent experiments have demonstrated in the breast tumor tissue the presence of biosynthetic and steroid-metabolizing enzymes capable of catalyzing the incorporation of [2,14C]acetate into [14C]cholesterol [1] and further oxidation at C-26 of [26-14C]cholesterol to 14CO2 and most probably cholangio acids [2]. Other investigations have shown that the particulate fraction isolated from involved lymph nodes metabolized [4-14C]cholesterol to [14C]pregnenolone and [14C]progesterone, recognized in many other endocrine systems [3]. There are other experimental studies that describe the ability of breast tumor tissues to transform [4-14C]pregnenolone to [14C]progesterone [4], [7α-{3H}]pregnenolone to [3H]dehydroepiandrosterone [5], [7α-{3H}]17α-hydroxyprogesterone to [3H]androstenedione [3], [7α-{3H}]dehydroepiandrosterone sulphate to [3H]dehydroepiandrosterone and [3H]testosterone [6], [4-14C]dehydroepiandrosterone to [14C]androstenedione [4], [1,2,6,7-3H]dehydroepiandrosterone to [3H]testosterone [5], [1,2,6,7-3H]androstenedione to [3H]testosterone

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[7], and [1,2,6,7-\textsuperscript{3}H]testosterone to [\textsuperscript{3}H]5a-dihydrotestosterone [8].

Several previous studies directed at determining the importance of aromatization as a source of estrogens have shown that breast tumor tissues can aromatize C19 androgens containing the A\textring, 3-keto grouping in Ring A of the steroid nucleus to aromatic C18 estrogens, as seen in the conversion of [\textsuperscript{14}C]-1 androstenedione to [\textsuperscript{3}H]testosterone [9, 10], [7-\textsuperscript{3}H]androstenedione to [\textsuperscript{3}H]estrone and [\textsuperscript{3}H]estradiol-17\beta [11], and [4-\textsuperscript{14}C]testosterone to [\textsuperscript{2}H]estradiol and [\textsuperscript{14}C]estradiol [4]. Other reports on in vitro studies have demonstrated conversions of [\textsuperscript{3}H]estradiol-17\beta to [\textsuperscript{3}H]estrone [7], [2,4,6,7-\textsuperscript{3}H]estrone to [\textsuperscript{3}H]estradiol [12], and [\textsuperscript{3}H]estradiol sulphate to [\textsuperscript{3}H]estrone and [\textsuperscript{3}H]estradiol-17\beta by human MCF-7 breast cancer cells [13].

When considered together the detection of necessary enzyme systems for the synthesis of C19, C\textring, C\textsuperscript{17}, C18 steroidal compounds may not be adequate for screening individuals or to establish a biological relationship of these metabolic events to breast neoplasia. In addition, previously reported studies have largely not attempted to determine the histological importance, as a way to quantitate severity and provide a measure of predictability for the physician of the biological behavior of the tumor. Most likely, intracellularly derived sex steroids may provide a specific local environment for metastatic aggressiveness for tumor progression from benign to undifferentiated invasive carcinomas. Tumor progression may also be accompanied by the elaboration of increased amounts of steroid hormones. Because progesterone exerts a strong proliferative effect on normal human breast ductal epithelium [14], we set out to test the ability of histologically proven infiltrating ductal carcinoma of the breast to metabolize pregnenolone to progesterone. This is very relevant to uncontrolled pathologic cell growth because in some species progesterone has been shown to stimulate DNA synthesis not only in the epithelium of the terminal bud but also in the ductal epithelium [15]. To gain insight into the properties of malignant cells, we sought to isolate, characterize, and quantitate [\textsuperscript{3}H]progesterone from incubations of the tumor homogenate with [\textsuperscript{7}n-\textsuperscript{3}H]pregnenolone, as substrate. It was thought that the present experiments would help define and assess the steroidogenic expression of the malignant tissue. Such information is likely to have theoretical and practical implications in the development of ‘suicide inhibitors’ for hormone-dependent breast cancer.

Materials and methods

Breast tumors from 7 pre- and post-menopausal women (one Caucasian, two Chinese, one Indian, and three Malays) aged 37-66 years were removed at surgery and examined for malignancy using the frozen section technique. When a malignancy was diagnosed, mastectomy was performed. Within 30 min of the mastectomy, the excised tumor was trimmed to remove any fat or grossly necrotic regions and was incubated within 1 h with radioactive precursor. Representative portions were removed to verify tumor histopathology. Only diagnosed primary infiltrating ductal breast carcinoma were used. Seven cases each having a histological diagnosis of infiltrating duct carcinoma were chosen for this study. The primary tumors had been staged clinically based on the Manchester classification [16]. Pregnancy was excluded in all cases.

The sample taken for incubation experiments was representative of the tumor as a whole. Homogenates were prepared by first mincing the tissue into small pieces and homogenizing with 10 ml of the buffer in an IKA-Ultra-Turrax (Staufen, Germany) instrument operating at maximum speed for 1 min.

Incubations were carried out with shaking in a water bath at 37°C for 3 h in Krebs-Ringer phosphate buffer, pH 7.4, using the tumor homogenate (6 g of tissue) divided equally into three flasks, each containing 1 ml of [\textsuperscript{7}n-\textsuperscript{3}H]pregnenolone (specific activity 11.0 Ci/mmol, Amersham International plc) purified by thin-layer chromatography in chloroform:diethyl ether (10:3, v/v) as substrate and supplemented with 1 mg of NADPH under an atmosphere of 95%\textsubscript{O}_{2}:5%\textsubscript{CO}_{2} in a final volume of 5 ml.

Controls were homogenates which had previous-