74

EFFECTS OF ESTRADIOL ON HISTONE BIOSYNTHESIS IN HORMONE-DEPENDENT AND HORMONE-INDEPENDENT HUMAN BREAST CANCER CELL LINES.

P. Bertrand-Mercat and J.R. Pasqualini,
C.N.R.S. Steroid Hormone Research Unit,
Foundation for Hormone Research,
26 Boulevard Brune, 75014 Paris, France.

It is well accepted that in hormone-dependent breast cancer, estradiol is one of the main factor of its growth by stimulating cell proliferation and DNA synthesis. Many mechanisms are involved in the regulation of DNA synthesis, including changes in chromatin structure. Histones can play an essential role in this structure. However, it is not clear how estrogens control chromatin protein synthesis, nor how this is related to the regulation of gene activity and cell replication. In the present study, we have examined the effects of estradiol (E_2) on histone synthesis in a hormone-dependent (MCF-7), and a hormone-independent (MDA-MB-436), breast cancer cell line. The cells were grown in MEM medium with 5% charcoal-treated fetal calf serum, then treated with 10^{-8}M E_2 during 2 days. [^{3}H]-Lysine was added (15 µCi/ml) and the incubation continued at 37°C for 6h. The histones were extracted with 0.4 N sulfuric acid and electrophoresed in polyacrylamide gels to separate the various components: H1, H2+H3, and H4. The incorporation of [^{3}H]-lysine was determined by laser densitometry and the specific activity expressed in dpm of [^{3}H]-lysine per mg of histones. In the MCF-7 cells, E_2 provokes a stimulatory effect of 29% ± 5.3 (SEM) on the synthesis of each histone related to the non-treated cells. In contrast, in the hormone-independent cell line MDA-MB-436, no effect was observed on the different histones. It is suggested that the selective stimulatory effect of E_2 on histone biosynthesis in the hormone-dependent breast cancer cell line could be related to the steps of the hormone response.

75

DEVELOPMENT OF NEW TRANSPLANTABLE HORMONE-RESPONSIVE RAT MAMMARY CARCINOMA MODELS.

S.A. Eccles, H.P. Purvies & A. Jarrett
Inst. Cancer Research, Sutton, Surrey, U.K.

Due to difficulties associated with the use of primary (DMBA and NMU-induced) rodent tumours, and their failure to mimic the malignancy of human breast cancer, we set out to develop a transplantable hormone-responsive tumour with reproducible growth and oestrogen sensitivity in syngeneic rats. From a tumour (induced by s.c. implantation of oestrone) which initially was only transplantable in oestrogen-supplemented rats, we produced two cell lines OES HR1 and HR2 which would grow under physiological endocrine conditions. Both tumours are hormone-responsive in that their growth is enhanced by oestrogen and totally inhibited in males or ovariectomised females; oestrogen receptors (ER) are stably expressed. Administration of oestrogen to male rats up to 250 days after cell inoculation produced rapid outgrowth of latent tumours, indicating that they are capable of prolonged dormancy under conditions of oestrogen deprivation. Tissue culture lines have been established and clonal heterogeneity of hormone responsiveness is being examined. The tumours are capable of haematogenous and lymphatic metastasis (Incidence 24-35%) and this is enhanced dramatically by oestrogen supplementation. These cells (and their hormone-independent sublines) will be useful to analyse critical factors in tumour progression.

76

RESPONSE OF TRANSPLANTABLE RAT MAMMARY CARCINOMAS TO ANTI-ENDOCRINE AGENTS.

A. Jarrett & S.A. Eccles
Inst. of Cancer Research, Sutton, Surrey, U.K.

The transplantable, oestrogen-responsive rat tumours (OES HR1 and HR2) and hormone-independent sublines derived from them (HI1 and HI2) have been compared for oestrogen receptor (ER) levels and their susceptibility to growth-inhibition by anti-endocrine agents. Using the dextran coated charcoal (DCC) assay, HR lines gave ER levels of 45-60 fmol/mg cytosol protein whereas HI lines gave low or negative values. In vivo both HR1 and HR2 have been shown to respond to Tamoxifen (2.4mg/kg) aminoglutethimide (32.3mg/kg) and 4-hydroxy-androstenedione (50mg/kg). Treatments were effective in inhibiting tumour growth whether initiated prior to tumour cell inoculation or during established tumour growth; however the former regime gave better results. The development of spontaneous metastases was also inhibited. Tamoxifen was shown to be oestrogenic when combined with ovariectomy; surgery alone completely prevented further tumour growth, addition of Tamoxifen restored growth rates to those seen in control animals. The growth and dissemination of HI tumours was unaffected by any drug treatment. These new HR cell lines will serve as a useful adjunct to the pre-clinical screening of novel anti-endocrine agents of all classes.

77

A METASTASIZING RAT MAMMARY TUMOR MODEL (EMR-86) FOR STUDYING HORMONE-DEPENDENT GROWTH KINETICS

J.H. Wijsman, J.H. van Dierendonck, R. Keijzer, C.J. Cornelisse and C.J.H. van de Velde
State University Leiden, Depts. of Pathology and Surgery, Leiden, the Netherlands

In order to improve treatment strategies for hormone dependent breast cancer, more knowledge is required on the *in vivo* interactions of hormones and growth factors with both tumor cells and surrounding stroma. Recently, we have developed a transplantable mammary cancer model in the Wag/Olac rat in which these interactions can be studied. This EMR-86 tumor is a well-differentiated ductal carcinoma of the comedo type which metastasizes to lungs and regional lymph nodes. The tumor only grows when stimulated by a s.c. implanted estrogen pellet, resulting in a tumor doubling time of 3-4 days. Using monoclonal antibodies against DNA-incorporated bromodeoxyuridine (BrdUrd), these rapidly proliferating tumors have a labeling-index (LI) of 18-25 %.
After pellet removal tumors rapidly regress. Only a small number of tumor cells remain, having almost completely stopped proliferating (BrdUrd LI <0.5 %). However, even after 6 months of dormancy, these quiescent tumors are able to grow out upon restimulation.
Since the estrogenic stimulus can be so easily manipulated, this EMR-86 model enables detailed study of s.c. transplanted tumors and their metastases during the hormone-induced transition from a quiescent towards an actively proliferating state and vice versa.