Dexamethasone modulation of in vitro growth pattern and of lung colonization ability in clones of a metastatic BALB/c mammary carcinoma cell line

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The expression of steroid receptors and the in vitro responsiveness to steroids were used to investigate the cell heterogeneity of a BALB/c mammary carcinoma cell line (TS/A) by means of its high- and low-metastatic clones previously selected in vitro. All the clones studied contained appreciable levels of receptors for oestrogens and for glucocorticoids. The in vitro responses of clones to 17β-oestradiol were very poor and comparable; conversely, a heterogeneous pattern of responsiveness to glucocorticoids was observed. In the presence of dexamethasone, the in vitro growth of high-metastatic clones was either unaffected or stimulated and dome formation was significantly increased. Dexamethasone treatment of low-metastatic clones caused inhibition of in vitro proliferation and a morphological shift from a fibroblast-like growth pattern towards the epithelial phenotype. One out of the three low-metastatic clones tested acquired the ability to form domes in the presence of dexamethasone, albeit sporadically. The in vitro treatment with dexamethasone significantly increased the lung colonization ability of the two low-metastatic clones studied, whereas no significant effect was observed with high-metastatic clones. Data presented here suggest that TS/A cell line consists of heterogeneous populations with peculiar proliferative and differentiative responses to glucocorticoids.

Introduction
Intratumour cellular heterogeneity in mammary carcinoma is a well-known phenomenon. In particular, it has been shown that several tumour lines consist of populations heterogeneous for cell morphology [2, 3, 6, 9, 17] and for metastatic capacity [2, 13, 15]. Moreover, there is evidence that hormone-responsive mouse mammary tumours are mixed populations of hormone-dependent cells with high oestrogen receptor content, and of independent cells with low oestrogen receptor content [11, 19]. Heterogeneity in hormone responsivity is likely to play an important role in determining the failure of endocrine therapy, and it could be involved also in the metastatic process [18]. However, cell heterogeneity for oestrogen receptor and its relationships to morphology, differentiation and metastatic capacity are still problematic [18]. Data obtained with different experimental models show that steroids can change cell morphology [21], and that a positive selection of hormone-independent populations with peculiar morphology can be obtained under unfavourable hormonal conditions [11]. These data suggest to take into account also the possibility that residual or altered responsiveness to steroid hormones could directly influence the morphological and/or the metastatic phenotype of single clones of neoplastic mammary cells.
We decided, therefore, to investigate cell heterogeneity in regard to the expression of steroid receptors and the in vitro responsiveness to steroids, and its relationships with the metastatic phenotype, in a new cell line (TS/A), which we have recently obtained from a spontaneous BALB/c mammary carcinoma [17]. This line has been shown to be heterogeneous in regard to cell morphology and metastatic ability [13]. Two sets of clones have been selected from TS/A agar cultures on the basis of colony morphology. All the clones studied were metastatic and the number of spontaneous lung metastases was correlated to cell morphology and to in vitro growth pattern: high-metastatic clones (E clones) displayed an epithelioid phenotype, whereas low-metastatic clones (F clones) exhibited some fibroblast-like features [13]. Since oestrogens and glucocorticoids are reported to play a central role in the morphofunctional development of mouse mammary gland [20], we tested TS/A line and its high- and low-metastatic clones for the expression of receptors and for the in vitro responsiveness to these steroids. The effect of glucocorticoids on the lung colonization ability has also been studied.

Materials and methods

Cells

TS/A cell line was derived from a spontaneous BALB/c mammary adenocarcinoma, which arose in a BALB/cAnNCrlBR female retired breeder [17]. The following clonal derivatives, selected in vitro from TS/A agar cultures [13], were used throughout the study: E1, E2, E3 (together referred to as high-metastatic E clones) and F1, F2, F5 (referred to as low-metastatic F clones). Cells were routinely cultured at 37°C in 5 per cent CO₂ in Dulbecco's MEM supplemented with 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin (referred to as DMEM) and with 10 per cent heat-inactivated foetal calf serum (FCS). All media constituents were purchased from GIBCO, Paisley, Scotland. TS/A cells used throughout the study were between the 20th and the 30th in vitro passage; clones' cells were between the 30th and the 50th in vitro passage after cloning.

Mice

Eight to 12-week-old BALB/cAnNCrlBR (referred to as BALB/c) female mice (purchased from Charles River, Calco, Italy) were used throughout the study.

Steroid receptors

Tumours induced in BALB/c females by subcutaneous injection of 10⁶ viable cells were removed 15–28 days after treatment and stored at -25°C. Steroid receptors were evaluated on the cytosol fraction, using a single saturating point in a dextran-coated charcoal binding assay. Determination of the oestrogen receptor content was performed according to the E.O.R.T.C. recommendations, with slight modifications [7], using 5 nM [³H]17β-oestradiol (Amersham International, U.K.; 95 Ci/mmol specific activity). Non-specific binding was determined in the presence of a 200 x excess of cold diethylstilbestrol (Sigma Chemical Co., St Louis, MO, U.S.A.).

Determination of the glucocorticoid receptor content was performed according to Goral and Wittliff [8]: 50 nM [³H]triamcinolone acetonide (NEN, Dreieich, Germany; 30–50 Ci/mmol specific activity) was used. Non-specific binding was