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Review

The human prostatic carcinoma cell line LNCaP and its derivatives

An overview

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Summary. The FGC (fast growing colony) line, a derivative of the LNCaP cell line shares all the main characteristics, including its androgen dependence, described for the original LNCaP cultures. A number of sublines originated from the FGC line which were characterized with respect to their response to steroid-depleted serum and to the synthetic androgen, R1881. After subcloning the FGC line a series of clones was isolated with distinct patterns of androgen-responsiveness. Among the sublines and clones studied, the FGC, FGC-JB and FGC clone-9 were androgen-dependent, whereas subline LNO, R and presumably also FGC clone-22 were androgen-independent. Distinct morphological differences were observed between the cells of the various sublines and between clone-9 and 22. The LNCaP cell line, its descending sublines and clonal derivatives provide a suitable in vitro model for studying different aspects of androgen-responsiveness of human prostate cancer.

Key words: Prostatic carcinoma - Cell lines - LNCaP Hormone-dependence - Androgens - Cell cloning

Introduction

Human prostatic cancer (PC) is a complex disease and the study of several aspects of this type of cancer can only be investigated by using appropriate animal and in vitro systems. In spite of the efforts of many investigators to establish in vitro cultures of prostatic epithelium, only a limited number of continuously growing cell lines of human prostatic carcinoma has been developed. Among the six permanent prostatic cell lines described up to now (Table 1) the LNCaP (Lymphe Node Carcinoma of the Prostate) cell line developed by Horoszewicz [10] from a metastatic lesion of a PC, is the most promising in vitro model of human PC, in particular because its growth is androgen dependent [11]. In addition, the LNCaP cells express prostate specific antigen and prostatic acid phosphatase (PAP) and contain androgen receptors. The production of PAP in cultures of LNCaP was shown to be regulated by androgens [11, 19].

Recently the LNCaP tumor model has aroused much interest. The LNCaP-FGC, which is a fast growing colony of an early passage of the original culture (Fig. 1), has been distributed from the Roswell Park Memorial Institute. This subline differs from the parental cell line only by its growth rate. Various aspects, such as the androgen dependent pattern of LNCaP growth, [1] the production and application of monoclonal antibodies against LNCaP [6, 7, 12], and the involvement of growth factors in the growth of LNCaP cells [20], are currently being studied in different laboratories.

Once established in the various institutes, the (long-term) cultures of the LNCaP-FGC cell line led to the development of a number of sublines with different patterns of hormonal responsiveness. Such sublines descended from the parental line either spontaneously or

Table 1. Prostatic tumor lines of human origin established in vitro

Tumor line	Established	Origin	Ref.
EB-33	1973	Primary tumor	[17]
DU-145	1975	Metast. (CNS)	[22]
PC-3	1976	Metast. (bone)	[15]
LNCaP	1977	Metast. (L.N.)	[10]
PC-93	1978	Primary tumor	[4]
TSU-PR1	1980	Metast. (L.N.)	[13]

CNS = central nervous system; LN = lymphe node

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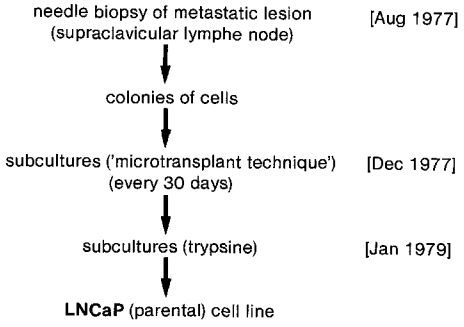


Fig. 1. Establishment of the LNCaP cell line, an in vitro model of human prostatic carcinoma [cf. ref. 10]

by maintainance of the original cell line in a medium with steroid-depleted serum. An example is the spontaneous occurrence of an androgen independent subline LNCaP-r (resistant) which was developed and further characterized by Hasenson et al. [8].

The Urological Department in Rotterdam has the disposal of all available LNCaP sublines. The aim of our investigations was to further characterize these sublines, with respect to their hormone responsiveness. This overview documents the derivation and major properties of the various LNCaP sublines and is partially based upon the results obtained in our own laboratory. Furthermore, this contribution is an introduction to the cytogenetical study of the LNCaP sublines performed in our institute and which is subject of a separate paper [16].

Material and methods

Cell lines

The LNCaP cell line was derived from culture explants of needle biopsy material taken from a lymph node metastasis of PC [11]. The culture was maintained only by passing colonies of cells using the technique of microtransplantation (Fig. 1) over a period in excess of one year. These cultures ultimately resulted in a cell line, LNCaP, that resembled a conventional cell culture, i.e. a line with a constant growth rate and that could be subcultivated by enzymatic dispersion [10].

Figure 2 shows a schematic representation of the LNCaP cell line and its derivatives as discussed in the present paper. The scheme also contains information on the current knowledge of the various sublines with respect to their androgen responsiveness. The LNCaP-FGC cell line was descended from a fast growing colony of the original LNCaP cultures (cf. Fig. 1). The FGC cell line was a gift of Dr. Horoszewicz (Buffalo, USA) and was transferred to our laboratory as a 16° passage culture of this line. The FGC cell line that was subsequently established at several institutes of Erasmus University in Rotterdam was designated with the suffix "GJ" (Fig. 2), but will be named FGC in the remainder of this paper.

The FGC-JB is a derivative of early passage cultures of the FGC. The LNO subline, also a gift from Dr. Horoszewicz, originated from a culture of an early (6°) passage of the parental LNCaP line that was grown and subsequently maintained in medium with steroid-depleted serum (Horoszewicz: personal communication). The LNCaP-r subline (designated R in our laboratory), was kindly provided by Dr. Hasenson (Huddinge, Sweden).

Cloning of the FGC cell line

Recently, by using the technique of limiting dilution it was attempted, to isolate possible preexisting clones of the FGC cell line. This was carried out with the 74th passage of the FGC line and resulted in a series of clones with distinct patterns of androgen responsiveness. Among these clones #9 and #22 (cf. Fig. 2) were partly characterized and some preliminary results are reported in the present paper.

Cell culture

The cell lines FGC, FGC-JB and R were maintained as monolayer cultures in RPMI medium (Gibco Europe, Breda, The Netherlands) supplemented with 10% fetal bovine serum (FBS; Boehringer, Mannheim FRG), 2 mM glutamine and antibiotics. The LNO cells were grown under the same culture conditions except that the medium contained 5% dextran-coated charcoal (DCC; dextran 0.1%, charcoal 1%) treated (i.e. steroid-depleted) serum. The untreated (FBS) serum contained 0.5–1.0 nM of testosterone (T), whereas in DCC serum less than 0.1 nM of T (the detection limit of the assay) was estimated.

All cells were grown in plastic tissue culture flasks (Falcon, Oxnard, USA). Cultures were kept in a humidified atmosphere of 5% CO2 in air at a temperature of 37°C. Cells were subcultivated at weekly intervals using a mixture of 0.05% trypsin and 0.01% EDTA.

Testing hormonal responsiveness

The growth of the different cell lines under standard condititons, i.e. in medium with 10% FBS, was compared with their behavior when FBS

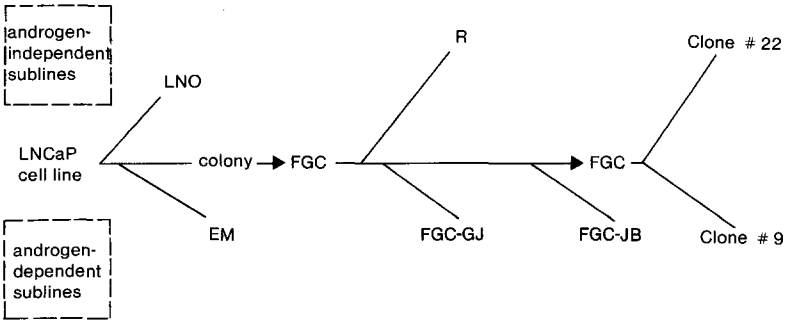


Fig. 2. Schematic representation of the LNCaP cell line and its descending sublines. EM: Electron Microscopically studied subline [cf. ref. 10]; LNO: Lymphe Node Original; FGC: Fast Growing Colony; FGC-GJ and FGC-JB: FGC-derivatives; R (= LNCaP-r): LNCaP-(androgen)-resistent [cf. ref. 8]

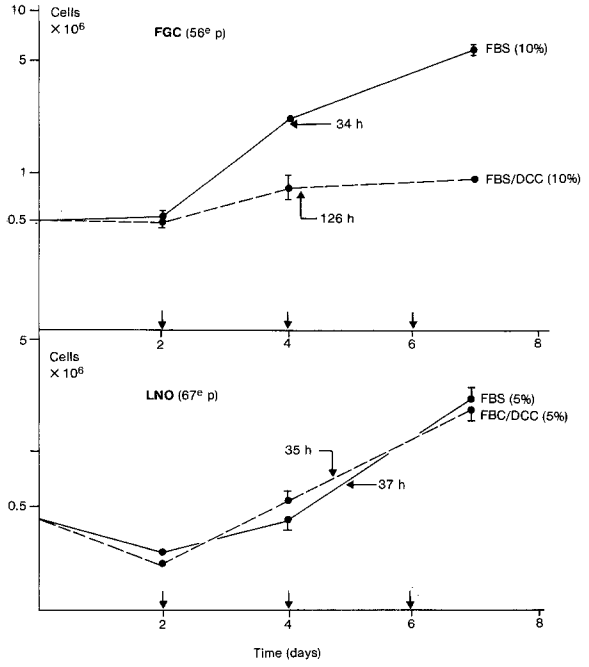


Fig. 3. Growth of FGC and LNO cells in complete (FBS) and in steroid-depleted (DCC) serum. ↓ = medium change

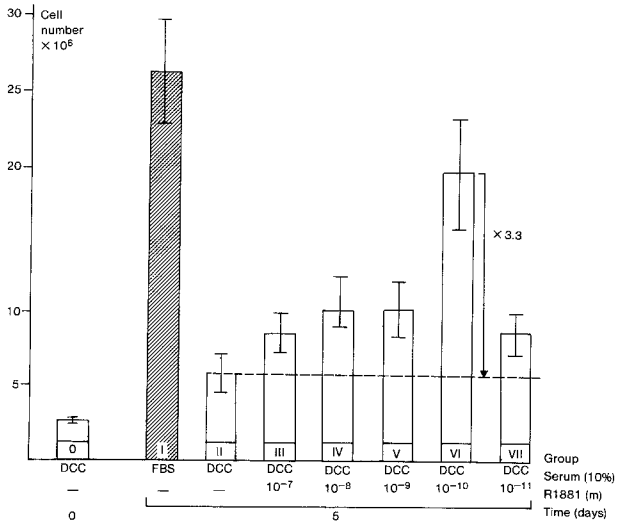


Fig. 4. Effect of different concentrations of the synthetic androgen R1881 on the growth of FGC cells cultured in steroid-depleted (DCC) serum. Data presented as mean ± S.D. ($n=4$)

was replaced by DCC serum. More specifically, the androgen responsiveness of the various cell types was determined by using the synthetic, nonmetabolizable [3] androgen R1881 (New England Nuclear, Boston, USA). Cells from stock cultures were plated at a density of 5.10^5/25 cm^2 flask and grown for 2 or 3 days in 5 ml medium containing DCC serum, whereafter the medium was replaced by medium containing R1881 in the range of 10^{-11} to 10^{-7} M. Control cultures were grown in medium with 0.01% ethanol vehicle. Media were changed every other day. Cells were harvested at 5, 6, or 8 days (see result section) after the androgen was added and counted using a hemocytometer.

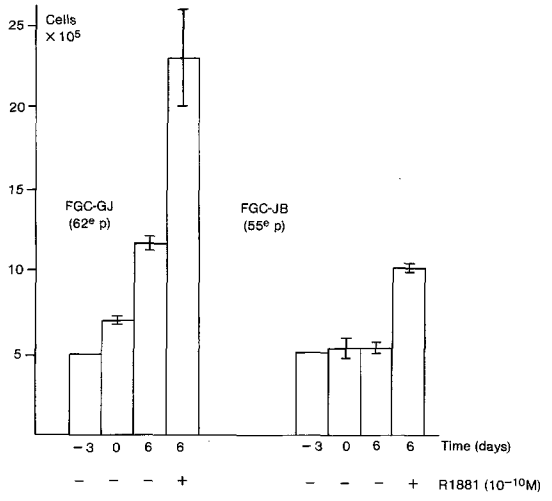
Results

Effect of steroid-depleted serum on LNCaP growth

Cultures of FGC and LNO differ in their response to medium supplemented with either FBS or DCC serum, as illustrated in Fig. 3. Both types of cells were cultured in medium with a percentage of serum in which the respective cells were routinely propagated (see Materials and methods). Moreover, no difference was observed between the growth of the FGC cell in medium supplemented with 10 or with 5 percent (FBS) serum (result not shown). After a lag phase of 2 days FGC cells exponentially grew for the next 5 days, the doubling time being 34 h, whereas during the same period of time in DCC medium the cells doubled only once (Fig. 3). When such cultures of FGC were continued in DCC serum, the cells eventually died after a number of population doubling times. A similar growth-inhibitory response upon steroid-depleted serum was found for cells of the FGC derivative, subline FGC-JB. By contrast, the LNO cells grew equally well in medium with either type of serum. The population doubling-time of LNO cells under both serum conditions was calculated to be approximately 35 h (Fig. 3). Similarly, the androgen-resistent cells of the R subline [8] could permanently be maintained in medium with 10% DCC serum.

Androgen responsiveness of the FGC cells

The ability of androgens (i.e. R1881) to stimulate growth of the FGC cells in the presence of 10% DCC serum is shown in Fig. 4. Cells were preconditioned for 2 days by growth in 10% DCC prior to exposure to either FBS or the R1881 containing DCC. The cells that continued to grow in DCC serum for 5 days showed a 2-fold increase in cell number (Fig. 4; group II versus 0). A statistically significant growth stimulatory effect was observed for all tested concentrations (range 10^{-7} –10^{-11} M) of R1881. Maximal response was seen at 10^{-10} M of the androgen, representing a 3.3-fold increase of the cell number over that in the DCC group (Fig. 4; group VI versus II). The growth-stimulatory effect of the optimal concentration of R1881 amounts to 75 percent of that found for cells grown in the presence of FBS (Fig. 4; group VI versus I). As 10^{-10} M R1881 was repeatedly found to be optimal



for growth stimulation, this concentration was chosen for further testing androgen-responsiveness of the various LNCaP sublines.

The FGC-JB line, which although like the parent FGC line was androgen dependent, exhibited a slightly different pattern of responsiveness. Generally, the growth of subline JB was less optimal: in comparison to the FGC line, JB cells detached more easily from the culture plastic and they had a slower growth rate. The JB cells, neither during a 3-day period of preconditioning, nor during the subsequent 6 days of culturing in DCC serum, showed no increase in the cell number (Fig. 5).

Fig. 5. Androgen-responsiveness of the FGC-GJ and the FGC-JB cells in the presence of medium with 10% DCC serum either or not supplemented with 10^{-10} M R1881

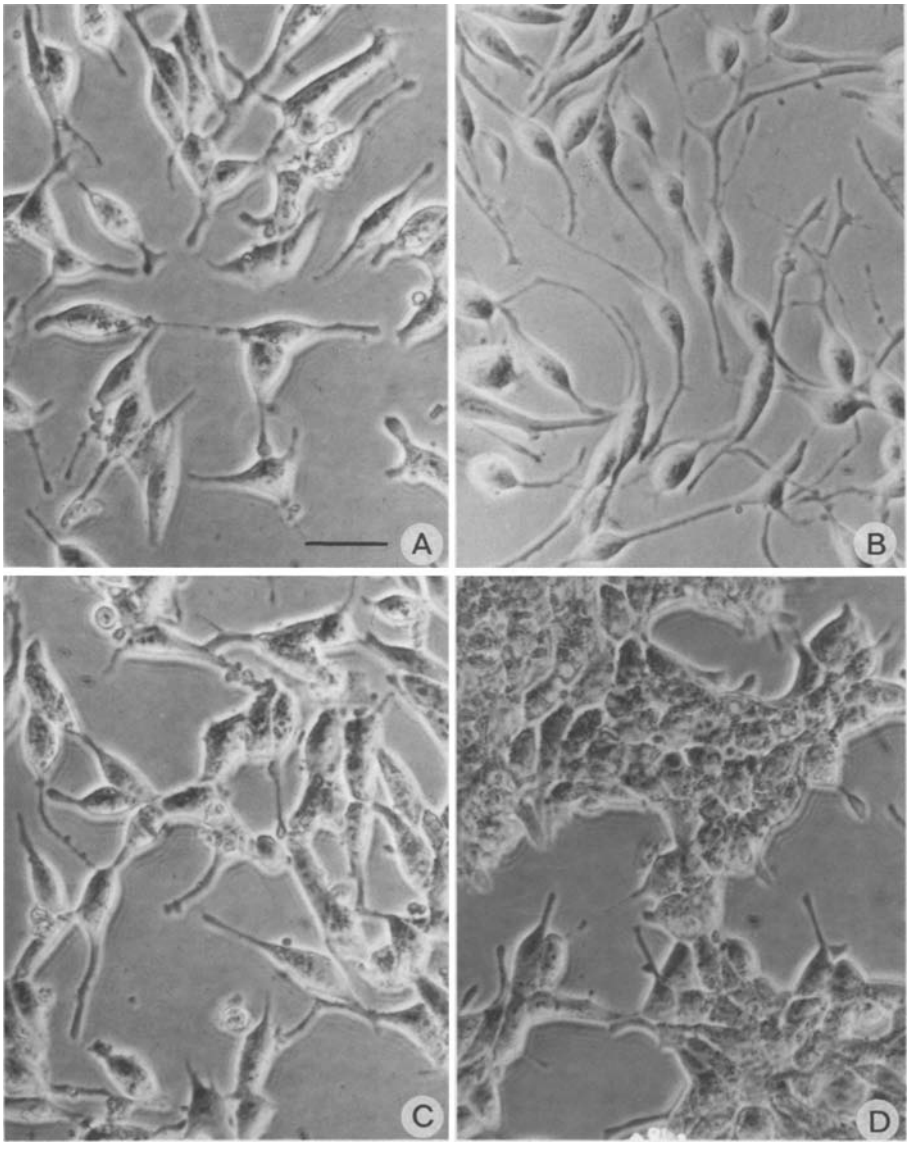


Fig. 6A–D. Morphology of the FGC cell line (**A**), the LNO line (**B**), and the from FGC descending clones 9 (**C**) and 22 (**D**), photographed under phase contrast; scale bar = 50 μm

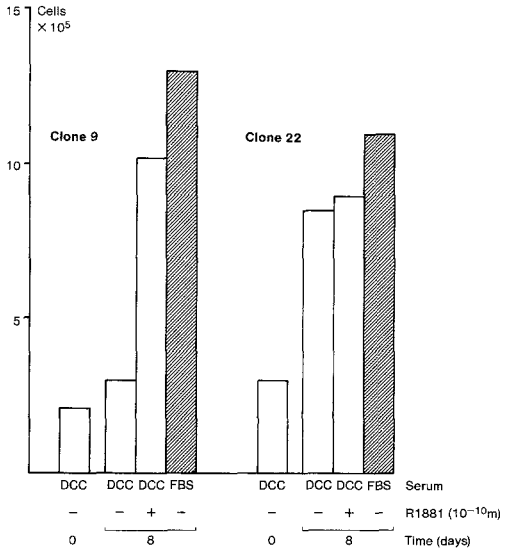


Fig. 7. Responsiveness of the growth of FGC clones 9 and 22 to FBS serum or DCC serum either or not supplemented with 10^{-10} M R1881

Independent of the baseline (no steroid) response to the DCC serum for both the FGC and the FGC-JB subline an approximate 2-fold increase of the cell number was obtained with R1881.

Characteristics of clonal derivatives of the FGC line

Attempts to subclone the FGC line resulted in a series of isolated clones with distinctive morphology and androgen-responsiveness. Among these subclones the cells of clones 9 and 22 were partly characterized. Morphologically the cells of clone 9 could hardly be distinguished from cells of the parent (uncloned) FGC line, as illustrated in Fig. 6. In contrast to the round and splindle-like shape of clone 9 and FGC cells (Fig. 6A and C), clone 22 consistently formed islands of cells with a flattened character as apparent in Fig. 6D. In addition, Fig. 6B shows the morphology of the LNO cell. In comparison to the FGC, the LNO cell is more roundly shaped and forms typical "high projections".

Distinctive patterns of serum- and androgen-reponsiveness were found for clone 9 and 22, as the preliminary data in Fig. 7 show. Cells of clone 9 did not grow in DCC serum, but their growth was stimulated by R1881 containing DCC serum and by complete FBS (Fig. 7; first panel). Hence, clone 9 consisted of the androgen dependent cell type. By contrast, growth of clone 22 was stimulated in medium with DCC serum irrespective of the addition of R1881 (Fig. 7), which meant that these cells may be androgen independent. Still, the cells of clone 22 exhibited a certain degree of androgen-sensitivity, as the addition of R1881 to the cultures changed the flattened shape of the cells to a more rounded appearance (result not shown).

Discussion

There has always been a need for the development of hormone-responsive model systems for human prostatic cancer. Examples of transplantable human prostate tumor in vivo are the hormone dependent PC-82 [21], PC-EW [9] and Honda [14] tumors. These well characterized tumors were hetero-transplanted directly into nude mice, but lack the property of continuous growth in vitro. Up to now LNCaP is the only androgen dependent prostatic cell line established in vitro. Hence, it offers the opportunity to study the involvement of (steroid) hormones and other factors in growth and differentiation of prostatic cancer cells in culture conditions.

The FGC line was directly descended from the LNCaP cell line that was initially developed from the patient material (Fig. 1). The FGC line, which retained all the main characteristics of the original LNCaP [11], was distributed widely from the Roswell Park Memorial Institute and it is the subject of an increasing number of different studies. During this time several sublines spontaneously arose from the FGC line, or have been obtained by subcloning the parental cell line.

The further application of the various LNCaP sublines should be preceded by their careful characterization, in particular with repect to the putative hormone dependent growth. It is recommended to carefully assess the appropriate culture conditions, especially when LNCaP cell lines have newly been established in any research institute. From our own experience as well as from other investigators it became clear that successful propagation of LNCaP cells strongly depends on the culture conditions, such as the serum (batch) used, the type of culture plastic, and the enzyme(s) used to subcultivate the cells. The cells also appeared to be susceptible to mechanical disruption.

DCC serum has extensively been used in studies on cell growth and response under influence of steroid hormones. The application of steroid-depleted serum, though essential for preconditioning the LNCaP cells in hormone-sensitivity tests of the present study, is limited as charcoal treatment not only removed steroid, but presumably also reduced the levels of growth factors and of lipoproteins in the serum. Hence, there is great need for defined media which allow more specific studies into the involvement of steroid and protein hormones or growth factors in the propagation and growth of prostatic epithelial cells and the LNCaP cells. For the studies of androgen-responsiveness of the LNCaP cell line non-metabolizable R1881 has been accepted the most suitable androgen as it was shown by Berns et al. [1]

Table 2. Growth properties of several sublines of the LNCaP in vitro model

Subline	Growth		Response to androgen (R1881)	Androgen receptor
	FBS	FBS-DCC		
LNCaP-EM	+	−	ND	ND
FGC	+	−	++	+
FGC-JB	+	−	+	+
LNO	+	+	+	+
R	+	+	−	+/−
FGC-clone #9	+	−	++	ND
FGC-clone #22	+	+	−	ND

FBS = fetal bovine serum; DCC = dextran-coated charcoal treated; ND = not determined
+ = growth; ++ = faster growth; +/− = reduced levels; − = no growth

that FGC cells metabolize T and dihydrotestosterone very rapidly.

The androgen dependent character of the original LNCaP cell line [11] and the FGC line [1] has been studied extensively and was also confirmed in our own laboratory, as illustrated in Fig. 3 and 4 of the present paper. By using a test system based on the enzymatic reduction of the tetrazolium salt MTT, Romijn et al. [18] also demonstrated the stimulatory effect of R1881 on the FGC cells. This rapid assay might be a promising alternative to time-consuming cell counting, moreover, it requires lower initial concentrations of cells.

Generally, when conclusions are drawn with respect to the androgen-responsive character of (prostate) tumor cell lines, at least the following cellular phenotypes can be distinguished: androgen-dependent, androgen-sensitive and androgen-independent. These cell types are defined as follows: androgen-dependent cells require a critical continuous level of androgen for maintainance in vitro. In contrast to androgen-dependent cells, which die without adequate androgenic stimulation (e.g. when cultured in DCC serum), androgen-sensitive cells grow faster in the presence of sufficient androgen but still can grow continuously even without the presence of androgen (e.g. in DCC serum). Therefore, such cells are androgen-independent but, as androgen-dependent cells, should still be indicated androgen-responsive. Finally, androgen-independent cells neither die nor decrease their growth rate under androgen-depleted conditions, consequently this cell type should be designated androgen-unresponsive.

The origin and background of the several sublines have been described previously. Like the parental FGC line, the response of the various sublines of FGC and two of its clonal derivatives to serum and to androgenic (R1881) stimulation has been assessed; the results are summarized in Table. 2. Considering the growth properties of the sublines, the FGC, FGC-JB and FGC-clone 9 were characterized androgen-dependent, whereas subline LNO, R and presumably also FGC-clone 22 are androgen-independent. Although not yet definitely established, the LNO subline appeared to be androgen-sensitive, as can be concluded from the observation that LNO cells grow in DCC serum (Fig. 3) but are still responsive to androgens (result not shown).

It should be emphasized that all the above-mentioned sublines are uncloned and consequently they probably consist of different subpopulations. Culturing these lines under different conditions may enhance the selection of particular androgen-dependent or independent cell types, which was supported by our experimental data, illustrated by the different behavior of the FGC-GJ and JB sublines in DCC serum (Fig. 5). Furthermore, it was demonstrated that different passages of the FGC line had a distinct pattern of androgen-responsiveness (result not shown).

By subcloning the FGC line an attempt to isolate clones with a more stable phenotypes was made. So far, two of the most promising clones were partly characterized and preliminary results showed a different response of these clones to steroid-depleted (DCC) serum and to R1881 (Fig. 7, Table 2). Interestingly, the lack of a growth response of clone 22 to androgen did not necessarily mean a lack of hormone response, as morphological changes were observed when the cells were cultured in the presence of androgen. Similar observations were reported for the prolactin response of clonal derivatives of the MCF-7 breast cancer cell line [2] and for the differential response of S115 mouse mammary tumor cells to steroids [5].

In conclusion, the LNCaP cell line and its descending sublines provide a suitable in vitro model for the study of several aspects of androgen responsiveness in human prostate cancer. Investigations into the role of growth factors, e.g. epidermal growth factor and its receptor [20], will further elucidate the mechanism of androgen stimulated growth of LNCaP cells. Comparative studies, such as the detailed cytogenetical characterization of the androgen-dependent and independent sublines of LNCaP [16], yield valuable information on the development of androgen resistance in human prostate cancer.

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