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Growth and differentiation of pluripotent embryonal carcinoma cells in the Snell dwarf mouse

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Summary To investigate the influence of hormones on the process of cellular differentiation the growth and differentiation of a transplantable tumour, induced by inoculation of pluripotent mouse embryonal carcinoma (EC) cells have been studied in athymic nude mice and, normal and hypopituitary Snell dwarf mice. All athymic nude mice developed tumours independent of the numbers of cells inoculated. In contrast, the tumour percentage in normal Snell mice was lower, showing a dose-dependent increase of takes. In dwarfs tumour percentage was comparable with that observed in normal Snell mice.

The morphological differentiation of teratocarcinomas grown in athymic nude mice, normal and dwarfed Snell mice shows derivatives of all three germ layers next to undifferentiated embryonal carcinoma cells. This suggests that the pituitary hormonal deficiencies of the dwarfs (growth hormone, thyroid stimulating hormone and prolactin) did not influence the tumour induction nor the development of the different tissues present in this type of tumour.

The influence of the pituitary on induction and growth of several neoplasms has been studied in hypophysectomized rats and mice, and in hypopituitary Snell dwarf mice (Ball & Samuels, 1936; Korteweg & Thomas, 1939; Piantanelli & Fabris, 1978). In Snell dwarf mice (Snell, 1929) a hormonal influence on tumourigenesis is suggested, since the spontaneous tumour incidence in dwarfs was low compared to normals. For the induction of tumours by chemical carcinogens differences in incidence between dwarfs and normals became apparent in some cases depending on the type of carcinogen used and the route of application. With regard to transplantable tumours in dwarfs, growth and the number of takes of Ehrlich ascites tumours and sarcomas 180 was comparable to that found in normals (Turolla, 1960).

In this paper we report on the growth of a transplantable tumour, induced by inoculation of pluripotent mouse embryonal carcinoma cells in Snell dwarf and control mice. EC cells are pluripotent cells as demonstrated by the formation of a teratocarcinoma after subcutaneous injection of a single EC cell and by the formation of chimaeric mice after the injection of EC cells into mouse blastocysts (Kleinsmith & Pierce, 1964; Brinster, 1974). The homology between EC cells and embryonic cells is further supported by similarities in immunological, biochemical, ultrastructural and developmental characteristics (Artzt *et al.,* 1973; Graham, 1977; Evans & Kaufman, 1981). These developmental characteristics provide the possibility of studying the influence of hormones on the process of cellular differentiation within such a tumour.

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Materials and methods

Experimental animals

Parent mice, heterozygous for the dwarf gene (dw/+), with unknown genetical background, were a kind gift of Prof. Tanner (Institute of Child Health, London) in 1973. Two couples were inbred over 11 years in our department and before that for several years in Prof. Tanner's department. Therefore it is very likely but not absolutely certain that the dwarf gene will be the only difference between Snell normals and dwarfs. In this stock immunological T-cell deficiencies are absent (Schneider, 1976).

Snell normal mice and dwarfs (males and females) were bred and kept under standardized laboratory conditions (Buul-Offers, 1983). The Snell normal mice used are either homozygous normal (+/+) or heterozygous for the dwarf gene (dw/+), because there are no phenotypic traits available for the recognition of both types of mice. Athymic nude mice (C57BL/10LP background) were obtained from TNO, Zeist, The Netherlands.

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Cells and culture conditions

PSMB is a pluripotent cell line cloned from tumour number OTT5568 (129/Sv mouse strain) (Stevens, 1970). The PSMB cell line was cultured on a feeder layer of STO cells. STO cells are thioguanine- and ouabain-resistant fibroblasts derived from a SIM mouse, isolated by Dr A. Bernstein (Ontario Cancer Institute). STO feeders were prepared by irradiation (Rö) of the cells with 30 Gy and seeded at $\sim 3.5 \times 10^6$ cells per 75 cm^3. PSMB cells (5×10^6) were seeded onto a STO-feeder and passaged every third day. All cells were grown in Dulbecco's minimal essential medium (bicarbonate buffer, glucose 4.5 $g l^{-1}$) supplemented with 10% heat inactivated foetal calf serum (FCS), 10^{-4} M β-mercaptoethanol and antibiotics (100 IU of penicillin and 0.1 mg of streptomycin ml^{-1} medium). The cells were routinely cultured in silicon rubber stoppered glass bottles. Cell cultures were split every two to three days by treatment for 5 min with Ca^{2+} and Mg^{2+} free PBS containing 1 mM EDTA and 0.25% trypsin. Cell lines were checked for mycoplasma by Hoechst staining (Chen, 1977).

Tumour growth and differentiation

Different numbers of PSMB cells (1.10^6–5.10^6) were inoculated s.c. in the neck of the experimental animals (age 9–11 weeks). Before inoculation the STO feeder cells were eliminated by passaging the PSMB cells once without feeder cells. The mice were inspected daily by palpation for the growth of tumours which were allowed to develop for periods from 7–100 days. The animals were killed under ether anaesthesia and the tumours excised. After fixation in 4% neutral buffered formaldehyde, tissue was embedded in paraffin and sections (5 μm) were stained with either H and E, Azan or alcian blue.

Results

In general tumours became detectable within 10 days after inoculation. Table I shows the number of takes; if animals did not develop a tumour within 3 months of inoculation they were scored as negative. The data show that all athymic nude mice develop tumours independent of the number of cells inoculated. In contrast, tumour percentage in normal Snell mice is lower, showing a dose-dependent increase of takes. In dwarfs the tumour percentage was comparable with that observed in normal Snell mice. Table II lists the tissue differentiations by PSMB cells in Snell normal, Snell dwarf and athymic nude mice. The morphological differentiation of teratocarcinomas grown in athymic nude mice shows derivatives of all three germ layers next to undifferentiated embryonal carcinoma cells. Tissue formation does occur in Snell normal and dwarf mice. In both mouse strains the formation of derivatives from all three germ layers is allowed without preference for, or selection against, any of the tissues observed on the athymic nude mice (Table II). Figure 1 shows some of the tissues formed in Snell dwarf mice.

Table I Tumour takes of PSMB cells inoculated in nude mice, normal Snell and dwarf mice

No. of cells inoculated	*No. of tumours/No. of mice*		
	N	*S*	*D*
1.10^6	10/10	2/10	0/10
3.10^6	10/10	10/19	
5.10^6	4/4	2/3	8/10

N: nude mouse; S: normal Snell mouse; D: Snell dwarf mice.

Animals were scored as negative when tumour development did not occur within 3 months of inoculation.

Table II Tissue formation in teratocarcinomas in nude mice, normal Snell and Snell dwarf mice

No. of cells inoculated		*Tissue types*			
		Neuronal	*Epithelial*	*Cartilage*	*Muscle*
1×10^6	N	++	++	+	±
	S	+	+	+	+
	D	+	++	++	++
3×10^6	N	+	++	++	+
	S	+	++	+	+
	D	++	++	+	+
5×10^6	N	++	++	+	±
	S	++	++	+	−
	D	++	+	+	±

N: nude mouse.
S: normal Snell mouse.
D: Snell dwarf mouse.
+: indicates the quantity of tissue.

Discussion

Teratocarcinomas are composed of different types of tissue. such as nerve tissue, muscle, epithelium and cartilage. Growth and differentiation of normal cartilage is controlled by a variety of hormones and metabolic factors amongst which are growth

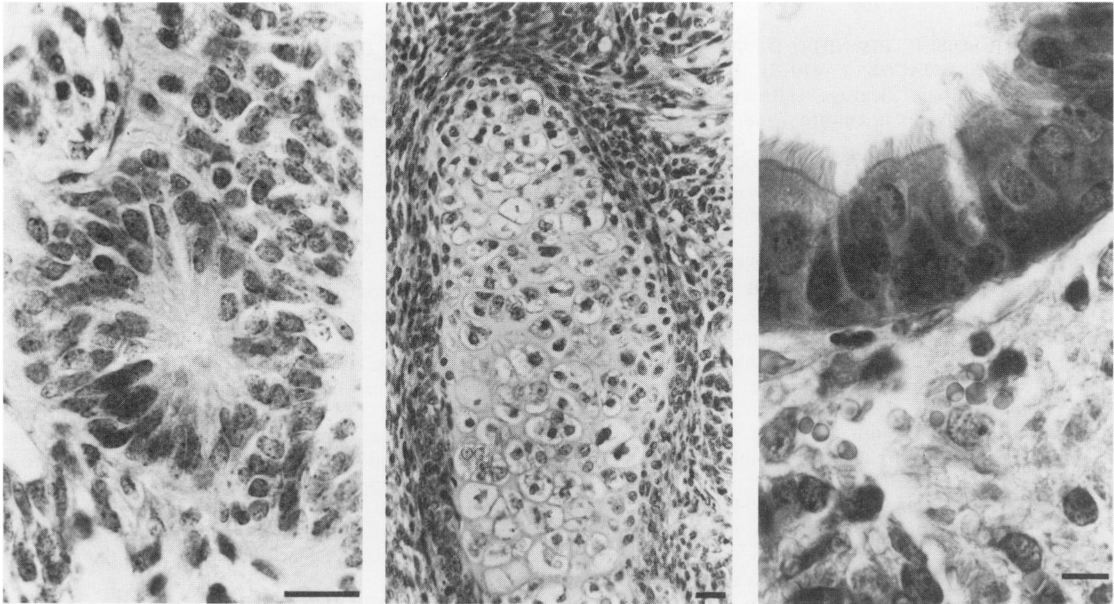

Figure 1 Morphological differentiation of a teratocarcinoma in a Snell dwarf mouse. Number of PSMB cells inoculated 3×10^6. Left; neuronal tissue (× 630), middle; cartilage (× 400), right; ciliated epithelium (× 1000).

hormone (GH)-dependent serum factors. These factors, called somatomedins, are mitogens which also stimulate amino acid transport and RNA and protein synthesis in different tissues (Salmon & Daughaday, 1957; Salmon & Duval, 1970; Buul-Offers, 1983).

It has been shown recently that amino acid transport and macromolecular synthesis, associated with the *in vitro* growth of a rat chondrosarcoma, were dependent on growth hormone-dependent serum factors as well as insulin (McClumbee & Lebovitz, 1980). Whether or not a similar hormonal dependency exists for the *in vivo* growth of this tumour is unknown.

In our *in vivo* system we found a similar percentage of tumour takes 3 months after inoculation in normal and dwarfed Snell mice, both of a lower level, however, compared to athymic nude mice. This suggests that the immunological systems of dwarf and normal Snell mice react in a similar way to counteract the development of this type of tumour. The large number of cells needed to generate tumours in these mice is probably necessary to overcome the immunological barrier due to difference in genetical background of the EC cells (129/Sv) and the Snell mice used. Undifferentiated mouse EC cells do not express H-2 antigens but do so upon differentiation (Jacob, 1977), therefore major genetic differences between the EC cells and the strains of mice used could have blocked the generation of differentiated tissues. Although EC cell transplantation within syngeneic strains of mice would have been preferable, the observed similarity in differentiation of teratocarcinomas in nude mice and dwarf and normal Snell mice indicates that no major immunological barrier exists. The similarity in morphological differentiation also suggests that the deficiency of dwarfs in growth hormone (GH) and consequently the somatomedians, thyroid stimulating hormone (TSH) and prolactin, did not influence the generation of the diversity of tissues present in this type of tumour.

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