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In vitro secretion of human chorionic gonadotrophin by bladder tumour cells

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Summary Human chorionic gonadotrophin (hCG) and alphafetoprotein (AFP) were measured in culture media from a panel of 29 cell lines including 9 bladder carcinomas, 5 normal bladder epithelia, 10 germ cell tumours, and 5 miscellaneous tumours and normal cell lines. In 7 of the 9 bladder carcinomas and 4 of the 5 normal bladder epithelia, the media contained hCG at levels ranging from between 34 and 3,600 IU/l. All other cell lines, including the 10 germ cell tumour lines gave negative results for hCG. These findings indicate that in vitro secretion of hCG is a common feature of normal and neoplastic bladder transitional epithelia, and support the hypothesis that parts of the genito-urinary epithelium have a potential for hCG production.

The high incidence of elevated levels of human chorionic gonadotrophin (hCG) and alphafetoprotein (AFP) in association with testicular germ cell tumours is well recognised in vivo (Seppala et al., 1986), but this phenomenon is relatively uncommon during culture of these tumours (Andrews et al., 1980). Ectopic production of hCG is also a well-recognised phenomenon in some non-gonadal epithelial tumours (Braunstein et al., 1973; McManus et al., 1976; Bellet et al., 1980). This is particularly common in gastric (24%), hepatic (17%) and pancreatic (50%) cancers (reviewed by Baylin & Mendelsohn, 1980; Heydeman et al., 1985; Seppala, 1986). The incidence may be even higher with malignant non-gonadal cells in vitro; in one such study (Rosen et al., 1980) 19 of 32 tumour cell lines secreted hCG (including one bladder carcinoma line). A few isolated cases of in vivo elevated hCG levels in serum and urine in association with bladder cancer have been reported (Civantos & Rywlin, 1972; Kawamura et al., 1978; Rodenberg et al., 1985; Norman et al., 1985). Very recently measurement of hCG has been used as a marker for monitoring metastatic bladder disease; 28 of 92 patients demonstrated measurable levels in blood (Dexexus et al., 1986). Immunohistochemical studies suggest that ectopic production of hCG is relatively unusual in bladder tumours with reports varying from 5 of 13 to 12 of 104 cases (Rodenburg et al., 1985; Shah et al., 1986). In the present study we have examined hCG and AFP secretion by normal and neoplastic bladder and testicular germ cell tumours in vitro.

Materials and methods

A total of 29 cell lines were examined in this study (Table 1). They included: 14 lines of urothelial origin (9 neoplastic (see Table 1), 5 normal); 10 germ cell tumours (gonadal) and 5 miscellaneous tumours and normal controls. The American Type Culture Collection (ATCC) and other lines held at the London Hospital were grown in a medium consisting of Leibowitz L-15 RPMI1640 (50:50, v/v) containing 20% heat inactivated foetal calf serum (FCS), transferrin (4 µg ml⁻¹), hydrocortisone (4 ng ml⁻¹), insulin (4 µg ml⁻¹), and penicillin-streptomycin (1,000 U ml⁻¹) (Gibco Ltd., Paisley, Scotland). Cell lines from the Institute of Urology were grown RPMI1640 plus 5% FCS (Gibco). Incubation was carried out at 37°C in a mixture of 95% air and 5% CO₂. The cell lines were grown to confluence in culture flasks (75cm² adherence area; Falcon Labware).

| Table 1 | Cell lines used in study. |
|------------------|-------------------------|
| **Cell lines**   | **Origin**   | **Source** | **Beta-hCG (IU l⁻¹)** | **AFP (U ml⁻¹)** |
| (A) Urothelial Lines |                        |            |                       |                   |
| T24              | TcE bladder       | ATCC       | <25                   | <10               |
| 382              | TcE bladder       | ATCC       | 56                    | <10               |
| RT4              | TcE bladder       | ATCC       | 34                    | <10               |
| TccSUP           | TcE bladder       | ATCC       | 34                    | <10               |
| ScaBER           | Soc bladder       | ATCC       | 2400                  | <10               |
| 5637             | TcE bladder       | ATCC       | 220                   | <10               |
| RT112            | TcE bladder       | ATCC       | 220                   | <10               |
| HT1376           | TcE bladder       | Inst Ur    | <25                   | <10               |
| TccDeS           | TcE bladder       | LH(1)      | 3600                  | <10               |
| (B) Germ Cell Tumours and Controls |            |            |                       |                   |
| GCT              |                        |            |                       |                   |
| GCT27            | GCT               | Inst Ur    | <25                   | <10               |
| 1618K            | GCT               | Inst Ur    | <25                   | <10               |
| HL               | GCT               | Inst Ur    | <25                   | <10               |
| 833K             | GCT               | Inst Ur    | <25                   | <10               |
| GH               | GCT               | Inst Ur    | <25                   | <10               |
| TERA II          | GCT               | ATCC       | <25                   | <10               |
| TERA I           | GCT               | ATCC       | <25                   | <10               |
| SuSa             | GCT               | Inst Ur    | <25                   | 18                |
| WG007            | GCT               | LH(1)      | <25                   | <10               |
| PJ077            | GCT               | LH(1)      | <25                   | <10               |
| Controls         |                        |            |                       |                   |
| 'Normal'         |                        |            |                       |                   |
| UV/K14           | Skin (keratinocyte)| LH(2)     | <25                   | <10               |
| Melme 3          | Skin (fibroblast)  | ATCC       | <25                   | <10               |
| Neoplastic       |                        |            |                       |                   |
| Wtu013           | Wilms tumour       | LH(1)      | <25                   | 13                |
| BrCaPE           | Breast carcinoma   | LH(1)      | <25                   | <10               |
| MJ003            | Breast carcinoma   | LH(1)      | <25                   | <10               |

Tcc = Transitional cell carcinoma. Scc = Squamous cell carcinoma. TcE = Transitional cell epithelium. GCT = Germ cell tumour. ATCC = American Type Culture Collection. Inst Ur = Institute of Urology. LH(1) = London Hospital originated by R. Iles. LH(2) = London Hospital originated by I. Leigh & P. Purks, Department of Dermatology.

NB. Cell lines NB/AJ, NB/UI, NB/U2 used at Passage 3 of origin and cell line TccDeS at pre-passage primary cultures.

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At this stage the medium (10 ml) was exchanged and the culture continued for a further 96 h. The medium was then harvested and, after removal of debris by centrifugation, was stored at −30°C until assayed. AFP was measured by radioimmunoassay (RIA) (Chard, 1978) and hCG by RIA directed to the beta subunit of this molecule (Norman et al., 1985). The minimal detection limit of the latter assay is 25 IU l−1.

Results

Media from 7 of the 9 bladder cancer cell lines contained detectable levels of beta-hCG (Table IA; Figure 1). In 2 of these (TccSUP and RT4) the concentrations were only marginally above controls but in the remainder the levels were substantially raised. Four of the 5 ‘normal’ bladder epithelial cell lines also showed levels of beta-hCG that were markedly elevated. The media from the germ cell tumours and other cell lines examined all gave negative results (Figure 1). Media containing hCG levels greater than 50 IU l−1 were also assessed using a semi-quantitative two-site immunoassay (Hybritech ICON). All gave negative results. Within the bladder cell lines there appeared to be a general association of epithelial morphology and high levels of beta-hCG (Table II). The occasional positive level of AFP (Table I) was only marginally different from the ‘noise’ level of the assay.

Discussion

There is good evidence that hCG may be produced by normal tissues (Yoshimoto et al., 1977, 1979b). Furthermore, using assays of exceptional sensitivity and specificity (i.e. as low as 0.01 ng ml−1), hCG can be detected in the urine of some normal non-pregnant subjects, post-menopausal females and women using oral contraceptive agents (Chen et al., 1976; Armstrong et al., 1984; Huang et al., 1984). Significant amounts of hCG (and other oncoplacental proteins) are also found in seminal plasma (Salem et al., 1984). These studies have led to the proposal that most tissues are capable of synthesising hCG, and especially some of the epithelia bordering the genito-urinary tract.

The present study confirms previous observations on the secretion of hCG by non-gonadal malignant cells in vitro (Rosen et al., 1980) and highlights the lack of in vitro secretion by the testicular germ cell tumour lines in long term culture (Andrews et al., 1980; Cotte et al., 1981). Given the basic ability of all cells to synthesise hCG and the derepression of this ability in many tumours (Yoshimoto, 1979b) it is not surprising that we have observed hCG secretion by bladder tumour cells in vitro. However, the secretion by ‘normal’ urothelial cell lines implies an innate capacity for hCG secretion. This is further supported by the apparent high frequency of this phenomenon (7 of 9 bladder carcinoma cell lines and 4 of 5 ‘normal’ bladder epithelia), especially when other cell lines, including the testicular germ cell tumours, were negative in this respect. If hCG secretion is a characteristic feature of bladder epithelial cells in culture, it would support the hypothesis that hCG secretion is a characteristic of the normal and neoplastic urothelium. The primary cell line TccDeS (beta-hCG 3,600 IU l−1) provides a link between the in vivo and in vitro situation. It was isolated from the pleural effusion of a patient with metastatic bladder cancer who had substantial levels of hCG in both blood and pleural fluid (760 and 2,700 IU l−1 respectively).

The apparent discrepancy between the secretion of hCG by germ cell tumours in vitro and in vivo may be a consequence of the very different micro-environment in vivo allowing the tumour cells to differentiate into trophoblast (Bronson et al., 1983; Vogelzang et al., 1983; McIlhinney, 1983). At the same time, a marginal increase of AFP does seem to be a feature of germ cell tumours in vitro (Table IB; Andrews et al., 1980).

We have not as yet attempted a full molecular characterisation of the ‘hCG’ detected in the cell media.

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**Table II**  
Bladder cancer cell lines – morphology and histology

| Cell line | Cell morphology                        | Grade | Stage | hCG levels (IU l−1) |
|-----------|----------------------------------------|-------|-------|--------------------|
| SCABER    | Large regular epithelial cells         |       | T3    | 2400               |
| 5637      | Small regular epithelial cells         | NR    | NR    | 220                |
| RT112     | Small regular epithelial cells         | I/II  | NR    | 220                |
| RT4       | Small irregular epithelial cells       | I/II  | T1    | 34                 |
| JR2       | Mixed epithelial and fibroblastic-like cells | III  | T3    | 56                 |
| TccSUP    | Mixed epithelial and fibroblastic-like cells | IV+  | T4    | 34                 |
| T24       | Mixed epithelial and fibroblastic-like cells | III  | NR    | <25                |
| HT1376    | Variable sized regular epithelial cells | III  | T2/3  | <25                |
| TccDeS    | Variable sized regular epithelial cells | I/III | T4    | 5000               |

NR = not reported. Histology survey: Hepburn & Masters, 1983; Masters et al., 1986. *Institute of Pathology, The London Hospital.
Nevertheless, the fact that high levels were determined by an assay which is specific for the beta-chain, while negative results were obtained with a two-site assay specific for intact hCG, strongly suggests that the material consists principally of the beta subunit or fragments thereof. It is interesting that of the five ATCC (1985 catalogue) cell lines listed as hCG secreters, 4 are of placental/foetal origin (3 choriocarcinomas and one line of SV/40 transformed normal placental cells) and one is a cervical epidermoid carcinoma. The latter non-embryonal tumour cell line (CaSk1) is reported to secrete the beta subunit than intact hCG (Pattillo et al., 1977). In a previous study, the hCG-like material found in urine and blood from patients with germ cell tumours appeared to be mainly intact hCG, while material from the patient with a metastatic bladder tumour consisted principally of free beta subunit (Norman et al., 1985). Variability of the relative concentration of intact hormone and free subunits, together with heterogeneous glycosylation of the protein chains, has also been reported in studies on patients with a variety of other tumour types (Weintraub & Rosen, 1973; Rosen & Weintraub, 1974; Kahn et al., 1977; Yoshimoto et al., 1979b). The degree of glycosylation affects various biochemical characteristics and also the rate of metabolism in vivo (Van Hall et al., 1971; Tsuruhara et al., 1972).

Independent production of subunit and intact hormone is not surprising as there is evidence that the genes for the alpha and beta subunits may be on different chromosomes. Thus, the alpha chain has been cloned (Fiddes & Goodman, 1979) and tentatively assigned to chromosome 6 (Naylor et al., 1984), whereas the beta chain, also cloned (Fiddes et al., 1980) has been mapped to chromosome 19 (Julier et al., 1984). Furthermore, the beta hCG 'gene' is itself very heterogeneous consisting of a cluster of at least eight genes arranged in tandem and inverted pairs; one of this cluster is the gene for the beta chain of luteinising hormone (LH) (Boorstein et al., 1982; Talmadge et al., 1983).

The significance of this genetic complexity is unclear at the present time (Talmadge et al., 1984; Whitfield & Kourides, 1985) but may partly explain the very variable results reported here and in the literature.

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