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Immunohistochemical localization of HCG and its subunits in testicular germ cell tumours*

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Summary. Native hCG and its α and β subunits have been localized by immunocytochemistry in 45 testicular germ cell tumours of the testis.

Positivity was found for the three molecules in areas with trophoblastic differentiation or in syncytial-like giant cells present in some seminomas. Isolated positivity of hCG α was demonstrated in isolated cells usually found in areas of entodermal differentiation of immature malignant teratomas, and probably of neuro-endocrine function.

This finding points to genomic derepression in tumour cells and probably also indicates a variability in subunit synthesis and a defect in subunit recombination.

Key words: Germ cell tumours – HCG – Subunits – Endodermal differentiation

Malignant germ cell tumours of the gonads are characterized in a substantial number of cases by the production of one or several tumour-associated antigens, i.e. alphafetoprotein (AFP) and human chorionic gonadotrophin (hCG) (Taylor et al. 1978; Bosman et al. 1980).

The latter marker is usually indicative of a trophoblastic differentiation. In early days, it was customary to discuss only the production of the native undissociated molecule (*t* hCG). Later it was discovered that hCG was composed of two subunits (Blackman et al. 1980; Gaspard et al. 1980b). The beta subunit is the marker of molecular specificity (and of biological activity) and is thus currently used in the hCG radio-immuno-assay of hCG. Now; isolated production and release of hCG has already been documented in normal placenta in organ culture (Gaspard et al. 1980a), in malignant pancreatic insulomas (Wahlström and Seppälä 1981) and in some breast tumours (Seppälä and Wahlström 1980). In normal pregnancy, there is also a simultaneous production of both subunits in a free state, together with the native molecule (Gaspard et al. 1980b).

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There is thus some evidence that production of hCG subunits and their recombination are differently coded and are also differently affected by pathological processes. These findings prompted us to look for a free production of hCG subunits in testicular germ cell tumours.

We report here the results of immunocytochemical work performed on a continuous series of 45 neoplasms and bring some evidence of an isolated production of hCG α in some composite tumours.

Material and methods

Forty five testicular germ cell tumours were diagnosed in our institution during the period 1979–1982. They were classified according to the WHO recommandations (Mostofi and Sobin 1977).

From each tumour, several blocks were selected, avoiding necrotic areas where possible. Serial sections were obtained and were routinely stained with haematoxylin-eosin, PAS and Grimelius.

Step sections were rehydrated and layered in a moist chamber with antisera directed towards hCG α, hCG β and native (i.e. recombined) hCG.

All antisera were used at a 1/1,000 dilution in PBS (0.05 M phosphate buffered saline, pH 7.4). Slides were incubated overnight. After a thorough washing with PBS, a classical PAP procedure was performed with 3–3′ diaminobenzidine as a chromogen and Harris haematoxylin as counterstain (Sternberger 1979). The antisera against hCG and subunits were raised in this laboratory and proved to be highly specific for their respective antigens (Reuter et al. 1976). Anti hCG α was extensively pretreated with an excess pure hCG (Serone-Rome) before being applied on slides; no cross reaction was noted neither with hCG β nor with the native molecule.

Control slides were incubated with antisera which had previously been run on a chromatographic column filled with Sepharose 4-B, activated with cyanogen bromide (Pharmacia – Sweden) and to which the selected purified antigen was covalently linked in amounts calculated to neutralize a working dilution of the antibody. Slides of normal first trimester placenta tissue were also simultaneously processed for the different immunocytochemical reactions and controls, in order to check the procedure on a well known positive tissue.

Results

The results of the immunocytochemical investigations are summarized in the table. The presence of native hCG together with both subunits was a characteristic feature of choriocarcinoma, of trophoblastic component of any germ cell tumour, and of scattered giant cells present in several cases of seminomas.

Arbitrary appreciation of the intensity of immunostaining showed that the native molecule usually gave the heaviest labelling, while hCG α positivity was less intense. Besides, as the immunostaining for each antigen was performed on strictly serial step sections, it was evident that native hCG and its subunits were colocated within the same cell.

Isolated presence of either native hCG or of its β subunit was never encountered. Positivity for native hCG and the β subunit or for native hCG and the α subunit was demonstrated in one case for each association. However, isolated positivity for both subunits, or more often for hCG α only was discovered with increased frequency in the group of immature malignant teratomas. The hCG α positive tumour cells were generally found in endodermal looking tubules (Fig. 1).

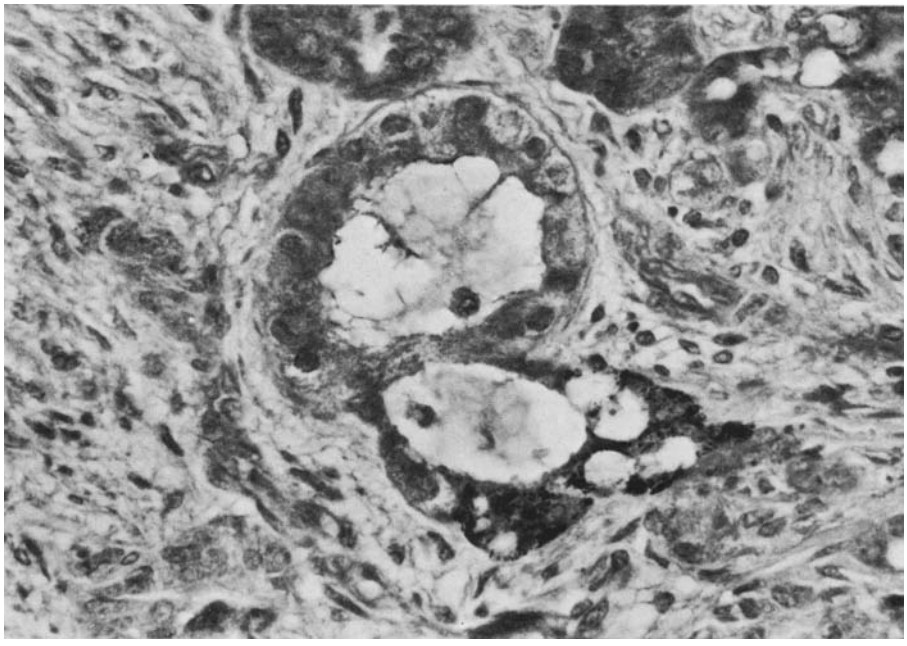

Fig. 1. Malignant immature teratoma. hCG α immunostaining (PAP + Harris haematoxylin counterstain $\times 400$). Several cells display cytoplasmic positivity

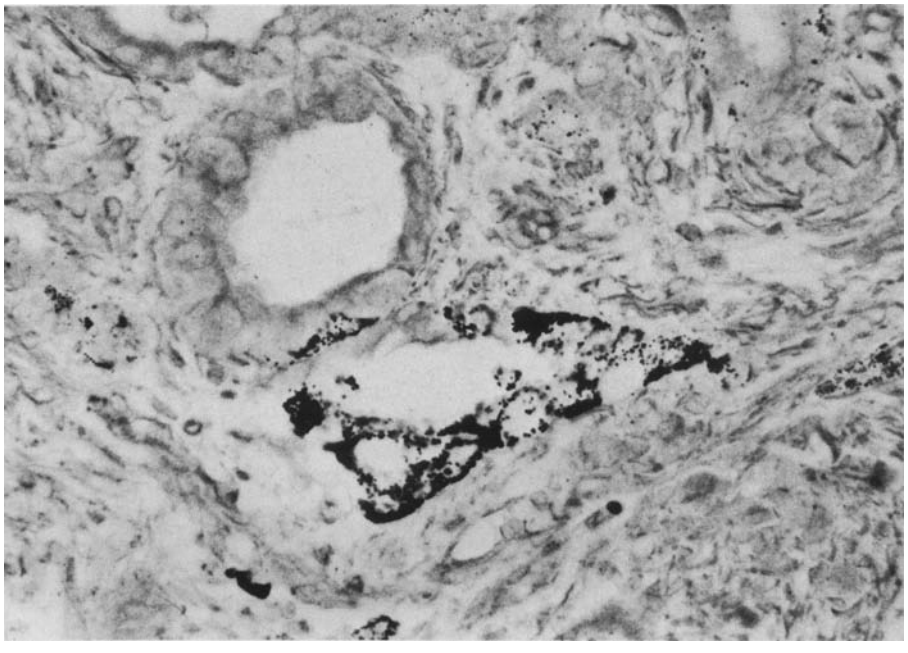

Fig. 2. Malignant immature teratoma. Grimelius stain. This is a serial section of that of fig. 1. The same group of cells are markedly argyrophilic ($\times 400$)

Table 1. The immunocytochemical results according to the tumour type

Immunocytochemical positivity	Number of cases				
	Seminomas	Mature teratomas	Immature malignant teratomas	Embryonal carcinomas	Chorio carcinomas
hCG + hCG α + hCG β	3	0	7	4	2
hCG + hCG α	0	0	1	0	0
hCG + hCG β	1	0	0	0	0
hCG	0	0	0	0	0
hCG α + hCG β	0	0	2	0	0
hCG α	0	1	4	1	0
hCG β	0	0	0	0	0
Total	4/11	1/3	14/19	5/10	2/2

Interestingly enough, most of these glands also possessed cells which were argyrophilic with the Grimelius stain (Fig. 2).

Thus in four out of nineteen cases of immature malignant teratomas, scattered hCG α positivity was the sole evidence of an incomplete tendency to hCG synthesis.

Discussion

When trophoblastic cells were conspicuous in a tumour, and in quite a number of isolated giant cells, native hCG and both subunits were identified in the same groups of cells. Moreover, with the use of serial thin sections, we could identify the reaction products within the same cell. This finding is quite in keeping with our earlier demonstration that normal placental trophoblast is immunoreactive fort native hCG and its subunits (Gaspard et al. 1980a). Thus tumourous trophoblast, i.e. foci of choriocarcinoma, must be able to synthetize the alpha and beta subunits, and to perform their recombination. This also holds true for the isolated giant cells frequently met with in seminomas or in teratomas.

It must be also noted that as in placental trophoblast, the immunopositivity was maximal for the beta subunit and native hCG while alpha subunit was associated with a lighter staining. We did not try to quantify the reaction products since too many variables are involved in the reaction processes. However all primary antisera were used at the same 1/1,000 dilution. We feel confident that there is in fact a lesser quantity of hCG α.

This discrepancy has also been reported for plasma values in cases of gestational trophoblastic neoplasia (Gaspard et al. 1980b).

The other important finding in this study is that in immature teratomas, isolated positivity for hCG α and hCG α + hCG β can be observed. This indicates that some tumour cells may be defective as regards the recombination process. Mann and Karl (1983) have demonstrated that there was free

subunit liberated in the plasma of 15% of their non-seminomatous testis cancer patients. Moreover these authors have demonstrated defective lectin binding of hCG produced by germ cell tumours: this finding is indicative of structural varieties of the molecule and particularly of variability of glycosilation. As complete glycosilation is necessary for secretion, this might indicate that in some cases, hCG or subunits are liberated only by cell death and tissue necrosis.

These findings may suggest an explamation for the absence of serum detectable hCG in cases where positive giant cells are seen.

Another hypothesis for free subunit production would be that some cells have undergone a gene derepression for alpha subunit synthesis. Such a phenomenon has already been demonstrated for placental cytotrophoblast in tissue culture (Gaspard et al. 1980a).

With regard to the isolated positivity of hCG α which is not infrequently liberated in the plasma (Mann and Karl 1983) we have demonstrated positive immunostaining in isolated cells. These are usually in parts of glands where step sections identify some argyrophilic cells. It seems evident that the glands are suggestive of endodermal differentiation (Brodner et al. 1980).

It has already been demonstrated (Öberg and Wide 1981), (Wahlström and Seppälä 1981) that alpha-subunit production was a salient feature of endocrine pancreatic tumours. Heitz et al. (1983) have stressed the preeminence of hCG α presence in malignant endocrine tumours of the pancreas and suggested that it could be a marker for malignancy.

In immature teratomas of the testis, isolated hCG α positivity was present in 4 cases/19 (slightly over 20%). Interestingly enough, it was also found in one case of embryonal carcinoma: in this specimen, hCG α positive cells enabled us to propose that the tumour was in fact a composite one, mixing embryonal carcinoma with elements of immature teratoma.

It is impossible to say at this time whether these findings may be of importance in the establishment of a prognosis. Further work is obviously needed in this respect.

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