

Subunits of Human Chorionic Gonadotropin: Unbalanced Synthesis and Secretion by Clonal Cell Strains Derived from a Bronchogenic Carcinoma

(ectopic hormones/cell culture/immunoassay)

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Communicated by Elkan R. Blout, March 9, 1973

ABSTRACT Three clones of ectopic hormone-producing cells from a single human neoplasm (bronchogenic carcinoma) show different rates of synthesis and secretion of chorionic gonadotropin and its alpha and beta subunits. For each clone, the amount of one or the other subunit always exceeded that of the complete hormone molecule. These findings may be analogous to unbalanced immunoglobulin chain synthesis in certain forms of myeloma.

The "ectopic" production of protein hormones and other polypeptides by malignant tumors of nonendocrine tissues is now well established (1-3). It has been proposed that this phenomenon is the result of derepression of the genome in the malignant cell, resulting in the transcription of DNA that is repressed in the normal cell. Such a mechanism predicts that the tumor cell product should have the same structure as that product produced by the normal cell of origin. This simple derepression hypothesis has been challenged by the occasional finding of "abnormal" tumor products, as measured by competitive radioassay techniques (4-6). However, because it is now recognized that certain polypeptide hormones may be synthesized in precursor forms (7) and, in addition, may be rapidly metabolized *in vivo* once they are secreted into the general circulation (8), immunological differences between "native glandular" hormone and the circulating peptide need not reflect differences in the primary structures of the polypeptide chains as they are synthesized in the cell.

It has recently been shown that the glycoprotein trophic hormones, follicle-stimulating hormone, luteinizing hormone, thyroid-stimulating hormone, and chorionic gonadotropin (hCG) are heteropolymers composed of two dissimilar subunits; an alpha subunit that is nearly identical among the four hormones and a beta subunit that confers biologic specificity (9). Thus, for these proteins, additional opportunities for production by a tumor of an "abnormal" product exist. For example, there could be unbalanced synthesis of the two subunits or faulty assembly of the subunits into the complete molecule. Indeed, the presence of hCG- β without accompanying complete hCG or free alpha subunit has been demonstrated in the plasma and in tumor extracts from a patient with pancreatic carcinoma (10, 11).

In order to examine whether isolated or unbalanced subunit synthesis observed *in vivo* might reflect metabolism of intact

hCG molecules and to study the mechanisms underlying control and expression of ectopic hormone production, we have been establishing such tumors in dispersed cell culture. We describe in this report our findings with three clonal strains of cells derived from a bronchogenic carcinoma previously shown to secrete biologically active hCG both *in vivo* and *in vitro* (12). The results of these experiments show that although the different clones each produce complete hCG molecules, they also synthesize and secrete larger amounts of at least one of the free subunits, and the ratios of subunits produced are greatly different among the clones.

MATERIALS AND METHODS

Establishment of hCG-Producing Clones. The clinical history of the patient has been described (12). In brief, he was 45 years old, had gynecomastia, and had widely metastatic hCG-producing bronchogenic carcinoma. At a time when the serum hCG concentration was 1.1 nM§ (40 ng/ml; normal <1.0), the serum concentration of hCG- β was <0.14 nM (<3 ng/ml; normal <1.0) and that of hCG- α was 1.7 nM (25 ng/ml)—although it was not clear whether the α -subunit had been secreted *de novo* or was derived from metabolism of complete hCG. With the patient's informed consent, a subcutaneous metastasis was excised and prepared for culture. The tissue was minced with scissors and incubated with 0.1% pancreatin solution (Viokase) for 8 min at 37°. The dispersed cells were concentrated by centrifugation, washed, and plated in 50-mm plastic tissue culture dishes (Falcon) in Eagle's Minimal Essential Medium with Earle's salts (Grand Island Biological Co.) supplemented with 5% horse serum and 5% fetal-calf serum. Cultures were incubated at 37° in a humidified atmosphere of 5% CO₂ and 95% air. Initially, fibroblasts grew briskly; however, after about 5 weeks in primary culture, an epithelial-like cell type appeared as the outgrowth from a minute fragment of adherent tissue. This second cell type grew slowly but relentlessly, and within 3-4 months had outgrown the fibroblasts. The epithelial-like cells (designated ChaGo) could be subcultured at low density (about 1000 cells per dish) and grown with almost no fibroblast contamination. When medium from such cultures was assayed (see below) for hCG and found to contain the glycoprotein (four

Abbreviations: hCG, human chorionic gonadotropin; hCG- α , the alpha subunit of hCG; hCG- β , the beta subunit of hCG.

§ The molecular weights of hCG (37,000), hCG- β (22,000), and hCG- α (15,000) were calculated from their amino-acid and carbohydrate compositions (13).

TABLE 1. Recovery of hCG added to culture medium

Incubation system	Duration of incubation (hr)	hCG added (ng/ml)	hCG measured (ng/ml)	Recovery of hCG (%)
Medium alone, no cells	0	10.0	10.0	100
	24	—	10.0	100
	48	—	10.0	100
	72	—	9.7	97
Diploid human fibroblasts*	24	10.0†	9.0	90
	72	—	9.7	97
Rat hepatoma†	24	10.0‡	10.0	100
	72	—	9.8	98
ChaGo*	24	10.0‡	10.1	101
	48	—	10.0	100
	72	—	9.4	94

* Each culture contained 0.40–0.50 mg of cell protein per dish. Endogenous production of hCG by ChaGo cells in these cultures was <0.55 ng/mg of cell protein per 24 hr.

† Each culture contained 1.5–2.3 mg of cell protein per dish.

‡ hCG (10 ng/ml) was added at zero time only.

control cell lines were tested and produced no detectable hCG), the epithelial-like cells were cloned. Three clonal strains of ChaGo cells were derived by plating single cells in Microtest II tissue culture plates (Falcon) with 100 μ l of culture medium. Such single cells grew, and each gave rise to a colony that was harvested, subcultured, and propagated in 250-ml culture flasks (Falcon). The three clones were designated ChaGoC₁, ChaGoC₅, and ChaGoC₁₀. They have been in continuous serial culture for 14 months.

The rate of growth of the cells (population doubling time) was determined as described for pituitary (14) and hepatoma (15) cell clones. Karyotypes were determined essentially as described by Sonnenschein *et al.* (16) 7 months after the clones had been established. Chromosomes in 25 metaphase plates of each clone were counted.

Measurements of hCG, hCG- α , and hCG- β . The intact hormone, hCG, and each of its subunits, hCG- α and hCG- β , was measured in culture medium and broken-cell preparations. The medium was assayed directly without fractionation. For intracellular hormone measurements, medium was removed from the dishes, the cells were washed 3 times with 0.15 M NaCl, and then scraped into distilled water and disrupted by sonic oscillation (17).

hCG, and its alpha and beta subunits, were determined by three independent radioimmunoassay methods; a second antibody was used to separate bound- from free-labeled hormone or subunit. hCG was determined by modification of our previously published method, using standard and labeled hCG rather than luteinizing hormone (18). hCG- α and hCG- β were determined by minor modifications of the methods of Vaitukaitis and coworkers (19, 20); for the *in vitro* studies reported here, each radioimmunoassay had the requisite degree of specificity to permit unequivocal quantitation without correction for the small degree of crossreaction.

In these experiments, production of hCG and its subunits is defined as the quantity of immunoreactive material that accumulates in the medium during a given period of time divided by the total cell protein at the time of collection (17). Cell protein was determined by the method of Lowry *et al.* (21). It is noteworthy that, under the conditions used, hCG was stable in culture medium both with and without cells (Table 1). This finding is of considerable importance, for it offers strong evidence that the hCG secreted from the cells in culture was not degraded in the medium and, therefore, that the subunits found in the medium were not derived from the extracellular breakdown of complete hCG molecules.

RESULTS

Characteristics of ChaGo cells

The data in Table 2 show that each of the ChaGo clones was aneuploid, although the epithelial morphology, as well as the numbers and distribution of chromosomes, were similar among the clones. The growth rate of all clones was relatively slow, with population doubling times of 97–121 hr. Each clone synthesized and secreted into the medium complete hCG molecules and subunits (see below) during both the logarithmic and early plateau phases (17) of cell growth. The intracellular concentrations of hCG, hCG- β , and hCG- α for clones C₁ and C₅ are given in Table 2. It is noteworthy that the intracellular concentration of at least one of the subunits was high relative to that of complete hCG. This disproportion was even more striking when extracellular concentrations of the subunits were measured.

Unbalanced subunit production

Cells of each clone were plated at moderate density (2×10^5 cells per dish) and were allowed to grow for about 3 weeks. Medium was changed twice a week and was collected from groups of replicate dishes for hCG and subunit assays. Cells were washed and total protein was determined. The rates of production of hCG, hCG- β , and hCG- α for two successive 3- or 4-day periods are shown in Figs. 1 and 2. For ChaGoC₁, considerably more hCG- β and α were produced than hCG. For ChaGoC₅, large amounts of hCG- α were produced relative to complete hCG, and hCG- β production was below the level of detectability of our assay method. For ChaGoC₁₀, the concentrations of hCG- β and α were more nearly equal but still exceeded considerably the production of complete hCG (Fig. 2). Similar results were obtained in two additional independent experiments of parallel design.

TABLE 2. Some characteristics of the ChaGo clones

Clone*	No. of chromosomes		Population doubling time (hr)	Intracellular hCG or subunit		
	Mode	Range		hCG (fmol/mg of protein)	hCG- β	hCG- α
ChaGoC ₁	58	48–61	102	3.2	73	16
ChaGoC ₅	58	49–61	97	<2.0	<50	110
ChaGoC ₁₀	57	51–60	121	nd†	nd	nd

* In all clones, cells were epithelial-like.

† nd = not determined.

DISCUSSION

Because hCG added to culture medium without or with ChaGo and other cell lines was essentially unchanged for at least 72 hr (Table 1), we conclude that the hCG- β and hCG- α measured in medium of ChaGo cells was not the result of extracellular degradation of hCG into its respective subunits. Furthermore, if hCG were being rapidly degraded into subunits intracellularly, we would expect that the relative proportions of α to β would be close to 1:1. That this is not the case is seen clearly in Figs. 1 and 2. These data lead us to conclude that there is disproportionate, unbalanced synthesis and secretion of the β and α subunits by ChaGo cells, and that the ratios of subunit production by the different clonal strains derived from the same tumor are different.

Since all clones produce some complete hCG, they must all produce both hCG- β and hCG- α , although, as we have shown, the ratios of subunit production vary markedly. It is of interest that the quantitative production of complete hCG by our clones derived from a bronchogenic carcinoma was far less than the production of hCG by clones derived from a choriocarcinoma (22). Our data suggest that this relative inefficiency derives in part from either unbalanced production of subunits or their faulty assembly.

These experiments do not allow us to conclude whether or not the structural genes for hCG- β and hCG- α are under the control of a single operator gene that became expressed during neoplastic transformation of the lung cell. There are, however, at least four testable possibilities that could explain our findings of differences among the clones: (a) abnormalities in DNA, (b) abnormalities in transcription or translation, (c) abnormalities in carbohydrate incorporation, and (d) abnormalities in subunit assembly to form complete hCG. In each case, these abnormalities may be qualitative (biosynthesis of an abnormal product) or quantitative (alteration in rate of synthesis or assembly of a normal product).

The unbalanced production of hCG subunits demonstrated *in vitro* does not necessarily imply similar unbalanced production *in vivo*. It is possible that tumor cells in culture behave differently from their counterparts in the host. Clearly the cultured cells are aneuploid (Table 2), a phenomenon that could well have occurred *in vivo* (23), although our ex-

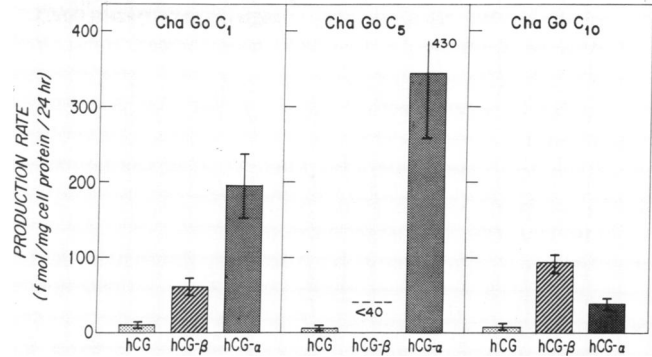


FIG. 2. Rates of production of hCG, hCG- β , and hCG- α by the same cultures as shown in Fig. 1 during an immediately subsequent 3- or 4-day culture period. Each bar gives the mean of 2-6 separate experiments and the brackets enclose the range. The dashed horizontal line for ChaGoC₅ gives the least amount of hCG- β that would have been detected by the assay.

periments do not rule out an *in vitro* change. That such a possibility should be considered is suggested by the observation of Gaspard and Franchimont (24) that, in short-term organ culture, fragments of human placental tissue released more hCG- α than hCG- β or intact hCG. However, the demonstration in our patient of a substantial excess of hCG- α relative to hCG- β in the serum indicates that at least part of this excess was derived from unbalanced production by the neoplasm in the host, for if the subunits had derived solely from hCG breakdown *in vivo*, they should have been present in equimolar amounts—unless the scarcer beta species were being cleared more rapidly. Actually, however, recent data in the rat show that the alpha species has the more rapid clearance of the two (25). Furthermore, the recognition in serum and tumor from two other patients of isolated production of the beta (10, 11) and of the alpha (Rosen, S. W. and Weintraub, B. D., unpublished data) subunit of hCG strongly suggest an *in vivo* counterpart to the data presented here. These arguments lead us to conclude that the findings made in the ChaGo cells in culture probably do relate meaningfully to the behavior of the tumor in the host.

The results of these experiments show clearly the variability of control of the expression of differentiated function in malignant cells. The functional heterogeneity of these cells is all the more impressive in view of the fact that they were derived from the same tumor and had similar morphologic, karyotypic, and cultural characteristics. Yet despite the differences in subunit production, each of these three clones contributed to the inappropriate production of complete hCG. The availability of clonal strains of such cells should permit the design of experiments to examine the mechanisms underlying the reappearance in adult neoplasms of the expression of previously stringently repressed genes.

We thank Drs. H. Hansen and F. Muggia for referring the patient, I. Stotler and A. McCammon for technical assistance with the radioimmunoassays, and Drs. J. Hickman, O. Bahl, and R. Canfield for gifts of purified hCG and its subunits. This investigation was supported in part by research grants from the National Institute of Arthritis, Metabolism, and Digestive Diseases (AM 11011) and the American Cancer Society (CI-65).

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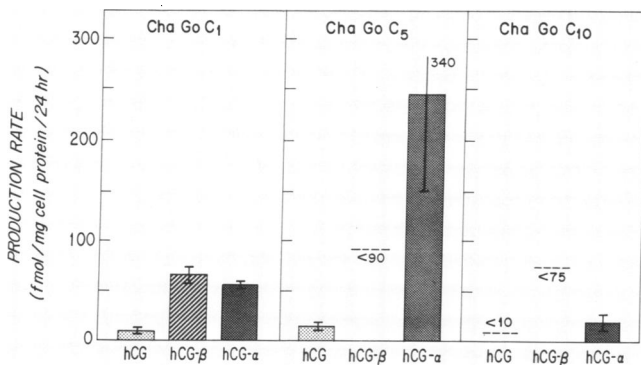


FIG. 1. Rates of production of hCG, hCG- β , and hCG- α by three clonal strains (ChaGoC₁, C₅, and C₁₀) of human bronchogenic carcinoma cells. Each bar gives the mean, and the brackets enclose the range of two separate tissue culture experiments; each experiment represents the mean of at least two radioimmunoassay values. The dashed horizontal lines give the least amounts of antigen that would have been detected by the assay.

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