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Possible Presence of an Embryonal Carcinoma-associated Proteoglycan in the Serum of Patients with Testicular Germ Cell Tumours

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THE TUMOUR markers alpha-fetoprotein (AFP) and human chorionic gonadotropin (hCG) are widely used in the management of testicular cancer and are rightly viewed as being of great value. They are not, however, products of the embryonal carcinoma malignant stem cell, but rather of other cell types which in some cases are non-dividing, non-malignant elements of the tumour. Furthermore, approximately 20% of patients with malignant teratoma do not have detectable AFP or HCG in their serum [1, 2]. We have previously reported that monoclonal antibody GCTM-2 recognises a keratan sulphate proteoglycan in the extracellular matrix of human embryonal carcinoma cells [3]. We have now developed a competitive-binding ELISA which demonstrates immunoreactivity with GCTM-2 in the serum of patients with testicular cancer.

Serum from 20 patients with active germ cell tumours was subjected to ELISA. A multiwell plate was coated overnight with a semi-pure preparation of GCTM-2 proteoglycan. Serially diluted samples of serum were mixed with GCTM-2 and applied to the wells. The binding of GCTM-2 to the wells was then detected using rabbit anti-mouse antibody conjugated to biotin, followed by streptavidin-horseradish peroxidase conjugate and 2, 2, azino-di'- (3-ethylbenzthiazoline sulphate) substrate, and the optical density at 501 nm was read. A reference solution of GCTM-2 proteoglycan was prepared by diluting semi-pure GCTM-2 proteoglycan in phosphate-buffered saline, and this was subjected to the same competitive-binding ELISA. A standard curve was then generated by plotting the logarithm of the antigen dilution against the corresponding optical density reading. The optical density readings of test samples were normalised to the baseline obtained from the standard curve, and then subjected to logit transformation. Those logit transform plots whose slope was at least 80% of that of the standard curve [4], and which were linear as judged by a correlation coefficiant of >0.9 were accepted as positives.

Seven out of the 20 patients sera (35%) yielded ELISA curves that were accepted as positives using the above criteria, one of these being in a patient who was AFP- and hCG-negative (Table 1). The antigen level was defined in arbitrary units as the antilogarithm of the serum dilution corresponding to a logit optical density reading of zero. Sera from 18 patients attending a thyroid clinic were tested as controls. None of these patients had a history of malignant disease. 1 out of 18 control sera (5.5%) also yielded a positive result with a level of 24 units; this came from a 68-year-old woman with Graves' disease who had positive antithyroid microsomal antibodies to a titre of 1 : 64 000.

The data suggest that GCTM-2 proteoglycan may be present in the serum of some patients with testicular cancer. Confirmation of this and explanation of the significant of the positive ELISA in one control patient will require a larger scale study.

Table 1. Patient characteristics and GCTM-2 antigen levels

Patient	Stage	Histology	Serum AFP kU/l	Serum hCG iU/l	GCTM-2 antigen units
1	IVCL2M+N+	MTU	46	28	121.5
2	IVCL3M+	MTI	9	8282	49.9
3	IVCL3	MTI/S	6	1085 023	Negative
4	IICM+N+	MTU	15 139	93 283	Negative
5	IVCL3Br+	MTI	3	184 600	Negative
6	IIIBN+	MTI	28	615	Negative
7	IVCL3Br+	MTT	<2	2042 848	Negative
8	Mediastinal	MTU	9168	<2	Negative
9	I	MTU	101	68	19.5
10	IIB	MTI	3302	<2	Negative
11	IIB	MTI	731	261	12.8
12	IIB	MTU	2	3	20.3
13	IVCL2M+	not biopsied	62	16 110	Negative
14	IVCL3M+H+N+	MTI	355	185	61 821.1
15	IVBL1	MTI	63	1746	Negative
16	IIB	MTU	340	15	Negative
17	IVAH+	TD/S	6	<2	Negative
18	I	MTT	52	<2	Negative
19	IVCL3	MTI	592	2	22.2
20	IVBL1H+	MTI	883	<2	Negative

MTU = malignant teratoma, undifferentiated; MTI = malignant teratoma, intermediate; MTT = malignant teratoma, trophoblastic; TD = teratoma, differentiated; S = seminoma.
Patients were staged according to the Royal Marsden Hospital staging system [5].
Histology was classified according to the British Testicular Tumour Panel [6].

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