Factor VIII as a marker of endothelial cells in follicular carcinoma of the thyroid

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SUMMARY Factor VIII-related antigen is a recognised marker of endothelial cells. A brief immunocytochemical study of its distribution in follicular carcinoma of the thyroid and its value in the recognition of vascular invasion by this tumour has been carried out. Ten cases of follicular carcinoma of the thyroid were studied. In each strong endothelial staining was found in the majority of vessels in the adjacent normal thyroid. Lymphatic endothelium was negative. In eight of the 10 cases the staining of vessels within the tumour was absent or very weak. Staining was also absent in the majority of vessels completely occluded by tumour, but was present in the endothelium of vessels only partly occluded by tumour. It is concluded that factor VIII-related antigen staining has only limited value in the recognition of vascular invasion in follicular carcinoma. The absence of vascular staining in the tumour leads us to suggest that inhibition of factor VIII production by the tumour could be a possible mechanism which facilitates vascular invasion and metastasis.

Factor VIII, an antihaemophilic factor, has three functionally distinct components: a clot-promoting factor, von Willebrand's factor, and a precipitating antigen, also known as factor VIII-related antigen (F VIII RAG).¹⁻³ Tissue culture studies have shown that F VIII RAG and von Willebrand's factor are synthesised by vascular endothelial cells,⁴ and immunoelectron microscopy has demonstrated F VIII RAG in human platelets, megakaryocytes, and endothelial cells.⁵ Recently, antibodies to F VIII RAG have been used in formalin-fixed, paraffin-embedded tissues to identify endothelial cells, and to establish the endothelial origin of a number of tumours of uncertain histogenesis. Lesions studied have included Kaposi's sarcoma,⁶⁻⁸ cerebellar haemangioblastoma,^{9 10} and haemangiosarcomas.^{7 8}

In thyroid pathology, there are two main areas where the localisation of an endothelial marker such as F VIII RAG might prove to be of diagnostic value—the definition of the entity of haemangioendothelioma of the thyroid, and the recognition of vascular invasion in follicular carcinoma. Malignant haemangioendothelioma is an extremely uncommon tumour of the thyroid, believed by some authors to be a variant of anaplastic carcinoma, and rarely recognised outside endemic goitrous areas.¹¹ We have

confined our attention in this brief study to the possible value of the use of F VIII RAG as an endothelial marker in the recognition of vascular invasion in follicular carcinoma of the thyroid.

Material and methods

Paraffin blocks of formalin-fixed tissues from 10 cases of follicular thyroid tumours with evident or suspected vascular invasion were selected. All cases were studied histologically by routine techniques including haematoxylin and eosin and orcein for elastic fibres. Immunoperoxidase technique was carried out to localise F VIII RAG. The immunolocalisation technique used was a modification of the DNP-hapten sandwich staining (DHSS) technique described by Jasani, Wynford-Thomas and Williams;¹² the detailed procedure is given elsewhere.¹³ The sections used to localise F VIII RAG were incubated in trypsin to ensure reproducible staining. Preliminary studies showed that this was achieved after 90 min incubation under standard conditions. The dinitrophenylated antiserum to F VIII RAG was used at a fixed dilution of 1/400. Positive control sections of lymph node and toxic diffuse goitre were included with each batch of staining for F VIII RAG, as were negative controls in which non-immune serum replaced the specific serum.

Results

ROUTINE HISTOPATHOLOGY

All 10 tumours studied had been diagnosed as follicular carcinomas because vascular invasion was considered to be present on haematoxylin and eosin stained sections, confirmed in some but not all cases by elastic techniques. The vascular invasion was present in extracapsular or capsular veins. Seven of the tumours were well differentiated, with a largely follicular pattern, three were less well differentiated, with a largely trabecular or solid pattern. The orcein technique stained the elastic of vessels outside the capsule, and allowed unequivocal identification of their nature. Vessels within or closely applied to the capsule were more difficult to identify, as their elastic structure was sometimes lost in extensive capsular elastic proliferation. Similarly, large vessel invasion by tumour well outside the capsule was easy to identify with certainty, while it was often more difficult to be certain whether rounded masses of tumour in the capsule were within vessels.

IMMUNOLOCALISATION

Positive staining for F VIII RAG was present in endothelial cells of blood vessels in control sections and in uninvolved thyroid tissue. In general, capillary endothelial cells showed the strongest positivity. As the diameter of vessels increased, the positive staining was weaker. This applied to both arteries and veins. The lining cells of normal lymphatic vessels were negative.

Endothelial cells of vessels within the follicular carcinoma were commonly negative, contrasting markedly with the positivity of endothelial cells of vessels of a similar size in the adjacent non-neoplastic thyroid tissue (Fig. 1). In only two of the 10 tumours was the overall staining of tumour vessels approximately equal to that of the vessels in the surrounding non-neoplastic thyroid. The vessels within the other eight tumours showed negative staining of the endothelium but in three of the tumours very occasional intratumoral vessels were positive.

When presumed vascular lumina outside the tumour were completely occluded by tumour, F VIII RAG staining was negative (Fig. 2), whether or not the structure could be identified as a vessel with elastic stains. In some vessels no endothelial-like cells could be identified, in others residual elongated cells with the morphology and location of endothelial cells were present, but were negative with F VIII RAG. When presumed vascular lumina, seen in the haematoxylin and eosin, were partially occluded by tumour factor VIII RAG staining was positive

Fig. 1 Follicular carcinoma of thyroid and adjacent normal gland stained for factor VIII RAG. The positive endothelial staining of the vessels outside the capsule (bottom) contrasts strongly with the absence of staining in the tumour vessels (top). DNP-hapten sandwich stain $\times 153$.

whether or not the structure could be identified as a vessel by elastic stains (Fig. 3).

Discussion

Several studies have stressed the value of the identification of extracapsular vascular invasion in the assessment both of the diagnosis and of the likelihood of distant metastasis in follicular carcinomas.¹⁴⁻¹⁶ While the recognition of such vascular invasion is sometimes easy, on other occasions it may be debatable. Factor VIII RAG has been shown to be a reliable endothelial marker in normal tissues in a number of studies,^{7 10 17} and has also been used to identify the vascular origin of various tumours. We have therefore in this study investigated the value of F VIII RAG immunolocalisation in the identification of vascular invasion in follicular carcinoma of the thyroid.

Our first finding, that the endothelial cells within thyroid tumours stained less well than the endothelial cells of vessels of similar calibre outside, was unexpected, although variable results had previously been found in endothelium from vessels within

Fig. 2 (a) Vascular invasion by follicular carcinoma, showing residual elastic in the wall. Orcein $\times 175$. (b) Serial section to (a) stained for factor VIII RAG. No endothelial staining is seen in the vessel occluded by tumour, compressed capillaries (arrowed) in the adjacent stroma show endothelial positivity. DNP-hapten sandwich stain $\times 175$.

Fig. 3 (a) Small vessel partially occluded by follicular carcinoma and surrounded by normal thyroid tissue. Haematoxylin and eosin $\times 132$. (b) The same vessel as (a) stained for factor VIII RAG and viewed at higher power. Most endothelial cells are positive in this vessel, which lacked demonstrable elastic. DNP-hapten sandwich stain $\times 280$.

tumours compared to those in the normal background tissue.^{7,8,10} We have no explanation for this difference—the possibility that this lack of staining may reflect the more recent origin of tumour vessels seems unlikely, for the vessels of reactive dermal proliferation have been shown to be positive.^{8,10} These tumour vessels do not resemble lymphatics, and erythrocytes were present in their lumina. We consider that proximity to the tumour may be associated with a loss of the antigen within the endothelial cells—either because of a change in the pattern of differentiation or to a humoral effect by the tumour. A mechanism that reduces the synthesis of factor VIII by endothelial cells, and disturbs the normal clotting response to vascular penetration by tumour might increase the chance that vascular permeation and metastasis could occur in that tumour.

The finding that vessels which were occluded by tumour did not show positivity for F VIII RAG, even though in some cases cells with the morphology of endothelial cells were present, was a disappointment from the diagnostic point of view. It is, however, consistent with the possibility that this antigen loss is due to proximity to the tumour. When spaces, presumed to be vessels on the haematoxylin and eosin stain, were partly occluded by tumour, the endothelium in the part not occupied by tumour was often well stained, and in these instances the use of F VIII RAG was of value in confirming the vascular nature of the structure.

We conclude that immunolocalisation of F VIII RAG has a limited place in the assessment of vascular invasion in follicular carcinoma of the thyroid. In many examples the use of an elastic technique was able to confirm that a major extracapsular vessel had been invaded. In a few instances the use of F VIII RAG immunolocalisation provided useful confirmatory evidence when a vessel was only partially occluded. However, if no elastic layer could be identified, it was still difficult to make the distinction between tumour which had penetrated the vessel wall and tumour which had merely invaginated the endothelial lining of a very thin walled vessel. The generally poor staining in tumour vessels in close proximity to tumour cells, and the reduction in staining in the endothelium of vessels occluded by tumour suggest that the tumour may induce a loss of F VIII RAG from endothelium. It is suggested that this may reflect a reduction in factor VIII synthesis, which represents a mechanism facilitating vascular invasion and metastasis.

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