

S100, α -smooth muscle actin and cytokeratin 19 immunohistochemistry in odontogenic and soft tissue myxomas

T Lombardi, C Lock, J Samson, E W Odell

Abstract

Aims—To compare the expression of S100 protein, α -smooth muscle actin (α -SMA) and keratin 19 in odontogenic myxomas and non-odontogenic myxoid lesions.

Methods—Formalin fixed, paraffin wax embedded tissue from seven odontogenic myxomas, three soft tissue myxomas, six hyperplastic myxoid dental follicles, two intramuscular myxomas, 12 cardiac myxomas, and seven normal dental follicles were examined immunocytochemically for S100 protein, α -SMA and cytokeratin 19 using the Streptavidin-biotin method.

Results—A minority of odontogenic myxomas (three of seven) were positive for S100 and the staining was of moderate intensity and in all myxofibroblasts. Soft tissue myxomas, normal dental follicles, intramuscular myxomas, and most enlarged myxoid follicles were negative. In the cardiac myxomas the cells forming cords and islands were positive in approximately half (seven of 12), but the dispersed stellate myxoblasts were positive in only two cases. A population of cells in all the odontogenic myxomas and hyperplastic dental follicles contained α -SMA, but such cells were sparse in cardiac myxomas and present in only four cases. Cytokeratin 19 was present in odontogenic epithelium of odontogenic myxoma and follicles.

Conclusions—A minority of odontogenic myxomas, but not other oral myxoid lesions, may express S100 protein and this could cause difficulty distinguishing myxoma from myxoid nerve sheath tumours. Sparse myofibroblastic cells occurred in all types of myxoma tested. The epithelium sometimes found within jaw myxomas expresses cytokeratin 19 and this is consistent with an odontogenic origin.

(*J Clin Pathol* 1995;48:759-762)

Keywords: Odontogenic myxoma, cardiac myxoma, cytokeratin 19, smooth muscle actin.

Odontogenic myxoma is a characteristic gelatinous, slow-growing, expansile benign jaw tumour. It presents most commonly during the second or third decade, may be slightly commoner in women and is considered to affect only the jaws, particularly the mandible.¹⁻⁴

The histogenesis and differentiation of myxomas are a subject of debate. The World Health Organisation and many authorities consider the

jaw myxoma to be an odontogenic tumour on the basis of its site, which is exclusive to the jaws,⁵ occasional association with missing teeth and the inclusion of islands of presumed odontogenic epithelium. It also has a close histological similarity to dental tissues such as follicle and papilla and other odontogenic tumours such as ameloblastic fibroma. However, this evidence is circumstantial and has been challenged⁴ because the appearances, whilst consistent with odontogenic ectomesenchyme, could also represent a more primitive fibroblastic or undifferentiated tissue.

Odontogenic myxomas are considered quite distinct from myxomas and fibromyxomas elsewhere in the body, many of which are thought to represent myxoid degeneration—for example, in nerve sheath tumours. A neural origin for cardiac myxoma is supported by immunohistochemistry demonstrating neural markers^{6,7} and pericellular basal lamina.⁶ Histologically, however, all myxomas are very similar and have no characteristic features to distinguish them. Differentiation of neural and odontogenic myxomas is problematic and there have been occasional reports of S100 positivity in odontogenic myxoma.⁸⁻¹⁰ We have therefore performed an immunocytochemical investigation of odontogenic myxomas, intramuscular and cardiac myxomas, and a variety of normal myxoid tissues with which myxoma is frequently confused by those unfamiliar with odontogenic lesions.¹¹⁻¹³ The aims were to ascertain the diagnostic value of routine S100 and α -smooth muscle actin (α -SMA) immunostaining in these tissues and by cytokeratin 19 staining to obtain further circumstantial evidence that the epithelium in jaw myxomas is odontogenic.

Methods

Archival formalin fixed, paraffin wax embedded tissue was retrieved and consisted of two normal tooth germs (gestational age 12 and 14 weeks), seven normal dental follicles, six myxoid enlarged follicles, two benign intramuscular myxomas, 12 cardiac myxomas, three soft tissue myxomas from the head and neck, one lesion from a case of oral focal mucinosis (oral equivalent to cutaneous focal mucinosis), and seven odontogenic myxomas. None of the cardiac myxomas was known to have been from a patient with familial myxoma syndrome (Carney complex). One of the cases of odontogenic myxoma has been described previously.⁸

Department of Oral
Medicine and
Pathology, Floor 28
Guy's Tower, UDMS
Guy's Hospital,
London SE1 9RT
T Lombardi
C Lock
E W Odell

Division de
Stomatologie et
Chirurgie Buccale,
Ecole de Medecine
Dentaire, Université
de Geneve,
Switzerland
J Samson

Correspondence to:
Dr E Odell.

Accepted for publication
20 December 1994

Summary of the immunohistochemical results

Lesion	Antibody		
	S100	α -SMA	Cytokeratin 19
Odontogenic myxoma	3/7	7/7	1/7 ^a
Soft tissue myxoma	0/3	1/3	1/3 ^a
Focal oral mucinosis	0/1	0/1	NEP
Enlarged myxoid follicle	1/6	5/6	5/6 ^b
Normal dental follicle	0/7	3/7	6/7 ^c
Intramuscular myxoma	0/2	0/2	NEP
Cardiac myxoma	7/12	4/12	NEP

^a Epithelium was present in only this one case.

^b Epithelium completely calcified in one case precluding assessment, scored negative.

^c No epithelium present (NEP) in the lesion, scored negative.

Sections were stained with haematoxylin and eosin and immunohistochemically using the Streptavidin-biotin peroxidase complex method (Dako, High Wycombe, UK). Representative, 4 μ m thick sections were dewaxed, rehydrated and endogenous peroxidases quenched in methanolic hydrogen peroxide before blocking non-specific binding in the corresponding non-immune serum. Antibodies (Dako) directed against S100 (polyclonal, diluted 1 in 300, 0.1 % trypsin 10 minutes), α -SMA (monoclonal clone 1A4, diluted 1 in 100) and cytokeratin 19 (monoclonal clone RCK 108, diluted 1 in 50, 0.1 % trypsin 10 minutes) were applied to each section for one hour at room temperature. Binding was visualised using biotinylated secondary antibody and the Streptavidin-biotin peroxidase complex developed with diaminobenzidine. Negative controls were performed by substituting primary antibody with non-immune serum of another species or phosphate buffered saline. Appropriate tissues were used as positive controls when no intrasectional control tissue was present.

Results

All myxomas were considered histologically typical. Odontogenic myxomas comprised stellate and spindle cells lying in copious myxoid matrix. Five of the seven lesions were fibromyxoid and also contained a small amount of finely fibrillar collagen. Rests or islands of odontogenic epithelium were present in only one case. Lesional tissue from the cardiac myxomas was closely similar or identical and no cases showed glandular differentiation.

Immunohistochemical results are summarised in the table. The myxoid tissues of the dental papilla and dental follicle in embryonic tooth germs, adult dental follicles and follicles showing myxoid enlargement were negative for S100, except for one myxoid follicle which showed a weak but generalised positivity. A variable number of α -SMA labelled cells were present in all but one of the enlarged myxoid follicles and in three of seven of the normal follicles. Cytokeratin 19 was found in the odontogenic epithelium of tooth germs, in the odontogenic epithelial rests of all normal dental follicles (fig 1) and in all the myxoid follicles which contained epithelium. In the negative case the odontogenic epithelium was completely mineralised.

In the odontogenic myxomas positive staining for S100 was found in three cases (fig 2). The staining intensity varied from weak to moderate and was present in all cells in two cases and had a patchy distribution in one case. Odontogenic myxomas, myxoid follicles and cardiac myxomas showed frequent α -SMA positive cells. In the odontogenic myxomas staining was of moderate intensity, but was generalised. In the enlarged follicular tissues there was more likely to be patchy staining or a more strongly stained outer layer and in the positively stained minority of the normal dental follicles the staining was also patchy. Cytokeratin 19 was consistently expressed by all the epithelium in the single case of odontogenic myxoma in which epithelium was present. All of the enlarged myxoid follicles and one normal dental follicle contained S100 positive nerve fibres. No nerve fibres were detected in any odontogenic myxomas by S100 staining.

Stellate or spindle cells in cardiac myxomas were positively stained for S100 protein in only two of the 12 specimens (fig 3) but a much higher proportion of positive staining was noted in the cords and islands of more condensed cells, which were immunolabelled in seven of

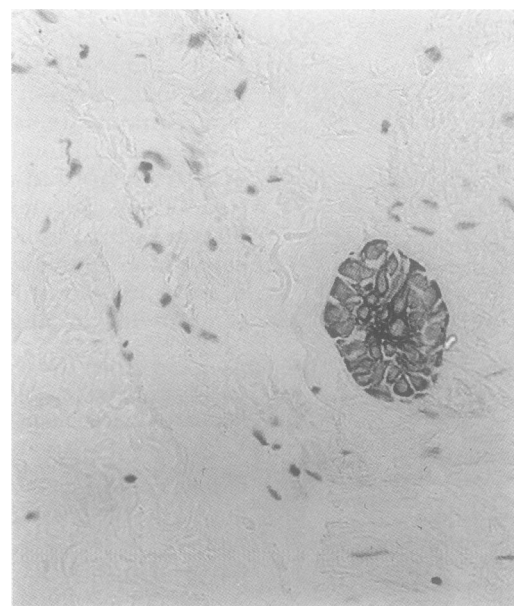


Figure 1 Dental follicle containing an island of odontogenic epithelium which shows strong immunopositivity for cytokeratin 19 in the sparse cytoplasm of the cells. Magnification $\times 312$.

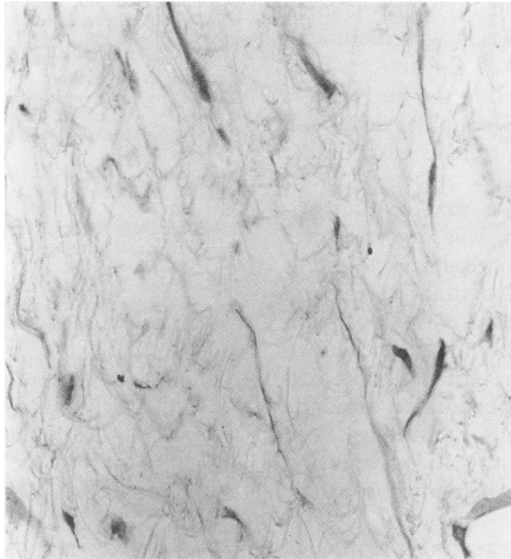


Figure 2 Odontogenic myxoma showing moderate immunopositivity for S100. Magnification $\times 488$.

12 specimens. S100 preferentially stained cells of the outer layers of the cords and basaloid cells of small tumour islands. Up to 50% of the cells were positive and the staining intensity varied from weak to strong. Four of the 12 cardiac myxomas contained sparse α -SMA positive cells but no glandular differentiation was present and all cases were cytokeratin 19 negative.

Discussion

Odontogenic myxomas are uncommon lesions which lack distinctive features and are easily confused histologically with other odontogenic tissues.¹¹⁻¹³ Their histogenesis is unclear but they are generally accepted to be distinct from other types of myxoma,¹⁴ some of which are thought to be variants of other, primarily neural, lesions.⁶ This histogenetic difference is not reflected in their histology and most myxomas appear very similar, if not identical.

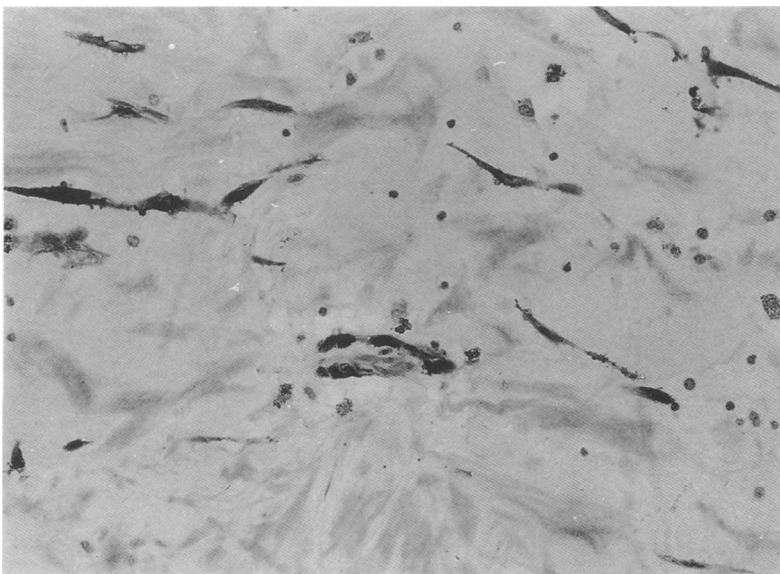


Figure 3 The larger cells of cardiac myxoma showing strong immunopositivity for S100. Magnification $\times 202$.

Differential diagnosis from neural myxoma depends on a diffuse rather than a lobular pattern, association with missing or unerupted teeth, absence of adjacent nerve, typical clinical and radiological features, and exclusion of other myxoid lesions.

There have been a small number of reports which have claimed S100 positivity in odontogenic myxoma,⁸⁻¹⁰ a finding which is disputed by others,^{15,16} but which could confuse the distinction between nerve sheath and odontogenic myxomas. Studies of the matrix glycosaminoglycans of odontogenic myxoma have shown little similarity to other odontogenic tissues.¹⁷

The results of this study show that S100 protein is not expressed in dental papilla and dental follicles, and are in agreement with previous reports on mouse¹⁸ and human myxomas.^{16,19} No S100 staining was found in enlarged myxoid follicles, with which odontogenic myxoma may be easily confused,¹¹ or in soft tissue myxomas. S100 positivity was seen only in a small minority of odontogenic myxomas but may be moderately strong and generalised. Unfortunately, even if positive, S100 staining cannot reliably differentiate myxoma from enlarged follicles because we found one follicle in which there was weak but generalised staining.

It was noticeable that S100 detected small nerve bundles in normal and enlarged myxoid dental follicles, as would be expected. However, no nerves were seen in the sections of odontogenic myxomas stained with S100. This is unexpected given the expansile gelatinous nature of the myxoma which envelopes surrounding tissues and while no definite conclusions can be drawn from this, albeit relatively large series, it appears that this feature might help distinguish myxomas from enlarged follicles. Nerve fibrils have not been reported in ultrastructural studies either.^{2,20}

The S100 positivity of odontogenic myxoma is not reproducible enough to be of value in differential diagnosis, but as an incidental finding it could cause confusion, favouring a diagnosis of myxoid nerve sheath tumour rather than myxoma. Luckily, although intraosseous neural tumours seem to have a predilection for the mandible,²¹ few are predominantly myxoid. The finding of S100 positive odontogenic myxomas raises the possibility that, like soft tissue myxomas, jaw myxomas are a heterogeneous group of lesions which cannot be distinguished histologically because of their relatively undifferentiated cellular phenotype.⁴ Distinguishing myxoid nerve sheath tumours from myxoma by electron microscopy is difficult because, although there is no basal lamina in myxoma, this structure is also sparse in nerve sheath myxoma and the appearances are essentially the same.²² We have attempted to detect basal lamina immunocytochemically using antibody to type IV collagen in myxoma but staining is uniformly negative irrespective of S100 positivity (data not shown). S100 positivity would appear to be incompatible with the suggestion that some jaw myxomas arise as variants of fibro-osseous lesions.⁴

The majority of cardiac myxomas stained positively for S100 and this is in agreement with previously published work.^{6,7} Positive staining was most frequently limited to the outer layers of cords and to islands of cuboidal cells, and was rare in the dispersed stellate and spindle cells.

Smooth muscle actin, a marker of myofibroblastic differentiation, was detected in a minority population of stellate and spindle cells all within the hyperplastic dental follicles and the odontogenic myxomas, consistent with a previous immunocytochemical¹⁶ and ultrastructural^{16,23} studies. One third of the cardiac myxomas examined contained similarly sparse α -SMA positive cells, as has also been reported previously,⁷ and this low proportion of cells is a common finding in many fibroblastic lesions.

Cytokeratin 19 was detected in the epithelial islands and rests within a single jaw myxoma and dental follicles. No glandular differentiation or epithelium was present in the cardiac myxomas. Cytokeratin 19 is expressed basally in non-cornified, stratified and many simple epithelia, and is not specific to odontogenic epithelium. However, all odontogenic epithelium and tumours derived from it are thought to express this cytokeratin²⁴ and this finding adds to the circumstantial evidence which suggests that the epithelium is odontogenic. It does not directly reflect the histogenesis of the myxoma because many myxomas contain no epithelium.²⁵

In conclusion, a minority of odontogenic myxomas and occasional enlarged myxoid follicles, but not normal odontogenic mesenchyme, may stain positively for S100 protein. This is important in differential diagnosis as S100 cannot be used to differentiate odontogenic myxoma from myxoid nerve sheath tumours. The finding that myxomas did not contain S100 positive nerve bundles bears further investigation as it may differentiate myxoma from enlarged myxoid follicle, a common diagnostic problem.

- 1 Barros R, Dominguez F, Cabrini R. Myxoma of the jaws. *Oral Surg* 1969;27:225-36.

- 2 Farman AG, Nortje CJ, Grotepass FW, Farman J, Van Zyl JA. Myxofibroma of the jaws. *Br J Oral Surg* 1977;15:3-18.
- 3 Slootweg PJ, Wittkamp AR. Myxoma of the jaws. An analysis of 15 cases. *J Maxillofac Surg* 1986;14:46-52.
- 4 Adekeye EO, Avery BS, Edwards MB, Williams HK. Advanced central myxoma of the jaws in Nigeria. Clinical features, treatment and pathogenesis. *Int J Oral Surg* 1984;13:177-86.
- 5 Dahlin DC, Unni KK. Bone Tumors. General aspects and data on 8542 cases. Springfield, Illinois: Charles Thomas, 1986.
- 6 Krikler DM, Rode J, Davies MJ, Woolf N, Moss E. Atrial myxoma: a tumour in search of its origins. *Br Heart J* 1992;67:89-91.
- 7 Johansson L. Histogenesis of cardiac myxomas. An immunohistochemical study of 19 cases, including one with glandular structures, and review of the literature. *Arch Pathol Lab Med* 1989;113:735-41.
- 8 Lombardi T, Kuffer R, Bernard JP, Fiore-Donno G, Samson J. Immunohistochemical staining for vimentin filaments and S-100 protein in myxoma of the jaws. *J Oral Pathol* 1988;17:175-7.
- 9 Nxumalo TN, Altini M. Myxofibroma of the jaws—a clinicopathological and immunocytochemical study. *J Dent Res* 1989;68:722.
- 10 Takahashi H, Fujita S, Okabe H. Immunohistochemical investigation in odontogenic myxoma. *J Oral Pathol Med* 1991;20:114-19.
- 11 Kim J, Ellis GL. Dental follicular tissue: misinterpretation as odontogenic tumors. *J Oral Maxillofac Surg* 1993;51:762-7.
- 12 Ang HK, Ramani P, Michaels L. Myxoma of the maxillary antrum in children. *Histopathology* 1993;23:361-5.
- 13 Dunlap CL, Barker BF. Diagnostic problems in oral pathology. *Semin Diagn Pathol* 1985;2:16-30.
- 14 Enzinger FM, Weiss SW. *Soft tissue tumors*. St Louis: Mosby, 1988.
- 15 Green TL, Leighty SM, Walters R. Immunohistochemical evaluation of oral myxoid lesions. *Oral Surg* 1992;73:469-71.
- 16 Moshiri S, Oda D, Worthington P, Myall R. Odontogenic myxoma: histochemical and ultrastructural study. *J Oral Pathol Med* 1992;21:401-3.
- 17 Slootweg PJ, van den Bos T, Straks W. Glycosaminoglycans in myxoma of the jaw: a biochemical study. *J Oral Pathol* 1985;14:299-306.
- 18 Dunlap C, Williams C, Barker B, Hof R. An investigation of S-100 protein in embryonic dental papillae of rats. *Oral Surg* 1984;58:575-8.
- 19 Lombardi T, Samson J, Bernard JP, Di Felice R, Fiore-Donno GMJ, Maggiano N. Comparative immunohistochemical analysis between jaw myxoma and mesenchymal cells of tooth germ. *Pathol Res Pract* 1992;188:141-4.
- 20 Simes RJ, Barros RE, Klein-Szanto AJ, Cabrini RL. Ultrastructure of an odontogenic myxoma. *Oral Surg* 1975;39:640-6.
- 21 Ellis GL, Abrams AM, Melrose RJ. Intraosseous benign neural sheath neoplasms of the jaws. Report of seven new cases and review of the literature. *Oral Surg* 1977;44:731-43.
- 22 Sist TC Jr, Greene GW Jr. Benign nerve sheath myxoma: light and electron microscopic features of two cases. *Oral Surg* 1979;47:441-4.
- 23 Hasleton PS, Simpson W, Craig RD. Myxoma of the mandible—a fibroblastic tumor. *Oral Surg* 1978;46:396-406.
- 24 Morgan PR, Shirlaw PJ, Johnson NW, Leigh IM, Lane EB. Potential applications of anti-keratin antibodies in oral diagnosis. *J Oral Pathol* 1987;16:212-22.
- 25 Ghosh BC, Huvos AG, Gerold FP, Miller TR. Myxoma of the jaw bones. *Cancer* 1973;31:237-40.