Pergamon

Oral Oncol, Eur J Cancer, Vol. 31B, No. 3, pp. 188–192, 1995

0964–1955/95 $9.50 + 0.00

0964–1955(94)00050–6

Expression of Tenascin in Hamster Buccal Pouch Mucosa During Experimental Carcinogenesis

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Experimental carcinogenesis by topical application of 7,12-dimethylbenz(a)anthracene (DMBA) in hamster buccal pouch mucosa was evaluated for expression of tenascin, an extracellular matrix glycoprotein expressed at the epithelial–mesenchymal interface during embryonic and fetal development, wound healing and in the stroma of various neoplastic lesions, by using immunohistochemical methods. The buccal pouch mucosa in normal hamsters showed immunoreactive tenascin either as a linear delicate band or without reactivity at the immediate vicinity of the basement membrane. During carcinogenesis, in the second to fourth week of application of DMBA, the hyalinous changes in the submucosal connective tissue had a weak but diffuse immunoreactivity for tenascin. The hyperkeratinised and hyperplastic mucosa following 5 weeks of application of DMBA showed focal areas of enhanced expression in the vicinity of the basement membrane. Subsequently, specimens showing hyperplasia, dysplasia, carcinoma *in situ* and invasive carcinomas had comparatively more widespread stromal immunoreactivity where the extent of enhanced reactivity positively correlated with the advancing lesion. These results compared with the results of expression in human normal mucosa, leukoplakia and squamous cell carcinoma of the oral cavity (Shrestha *et al.*, *Oral Oncol, Eur J Cancer* 1994, 30, 132–137) suggest that the expression of tenascin in experimental carcinogenesis of hamster buccal pouch mucosa, as a model, faithfully mimics the same in human oral mucosa.

Keywords: oral cancer, tenascin, carcinogenesis

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INTRODUCTION

HAMSTER buccal pouch mucosa, following experimental carcinogenesis using 7,12-dimethyl-benz(a)-anthracene (DMBA), is a most widely studied animal model to study events involved in the genesis of precancer and cancer of the oral mucosa in humans [1]. An appropriate model system for basic research in carcinogenesis is a prerequisite to understand the processes in the pathogenesis and subsequently, prevention and treatment of cancer.

Tenascin, an extracellular matrix protein, is expressed in the mesenchyme at sites of epithelial–mesenchymal interactions during embryogenesis, wound healing and in the stroma of several human tumours. It may have a role in cell adhesion and motility, guidance along cell migration pathways, shedding of epithelial cells from surfaces, promotion of cell growth, demarcation of tissue boundaries and tissue modelling [2–10]. Interactions between tumour cells and extracellular matrix are of importance in tumour invasion and metastasis, and the expression of tenascin, particularly at the interface of epithelial tumour cells and the surrounding mesenchyme, may suggest an altered cell-matrix interaction that may facilitate epithelial tumour cell invasion during carcinogenesis. An enhanced tenascin immunoreactivity in leukoplakia and squamous cell carcinoma of the oral cavity has been reported [11]. The object of the present study was to evaluate expression of tenascin during experimental carcinogenesis in hamster buccal pouch mucosa and to further characterise these neoplasms as a possible model of human oral precancer and cancer in terms of tenascin expression.

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Received 1 Sep. 1994; provisionally accepted 25 Oct. 1994, revised manuscript received 22 Nov. 1994.

MATERIALS AND METHODS

The investigation used 36 male 2-month-old Syrian golden hamsters. The cheek pouches were painted three times weekly using a no. 4 sable brush with 0.5% 7,12-dimethylbenz-(a)anthracene (DMBA) in mineral oil. The hamster buccal pouch mucosa were observed with the naked eye throughout

the experiment and three hamsters at a time were sacrificed to obtain the specimens every week from week 0 to 13. The buccal mucosa from the hamsters of a corresponding age group were taken as normal controls.

The specimens were fixed in Bouin's solution for 3–5 h and embedded in paraffin. Sections of 4 μm were stained using haematoxylin and eosin for histopathological evaluation and immunohistochemical studies were carried out using monoclonal antibody anti-tenascin MAB 1927 (1:500; Chemicon, California, U.S.A.), a three stage streptavidin biotin immunoperoxidase technique, and the chromogen 3,3′-diaminobenzidine. The details of the procedure have been described elsewhere [11].

RESULTS

Normal mucosa

The normal hamster buccal pouch mucosa, composed of 3–4 layers of squamous cell epithelium with a layer of small cuboidal basal cells without rete peg formation (Fig. 1A), showed a thin delicate band-like immunoreactivity (Fig. 1B) or no reactivity of tenascin in the immediate vicinity of the basement membrane but with a weak diffuse reactivity in traces in the submucosal connective tissue (Fig. 1C). There were areas of positive and negative immunoreactivity in different areas of the same specimen.

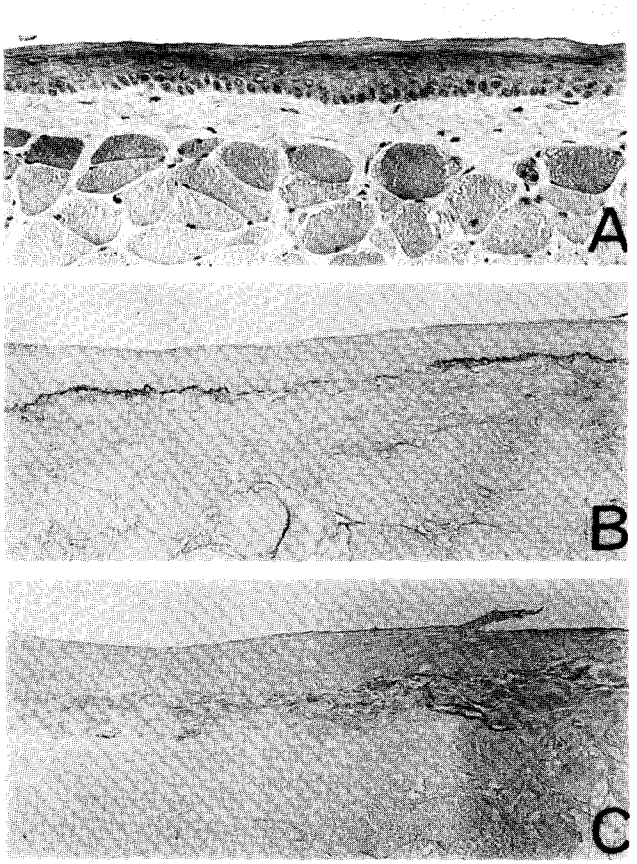


Fig. 1. Haematoxylin and eosin stained normal hamster buccal pouch mucosa (A). Tenascin immunostaining: the epithelial–connective tissue interface shows areas of a thin delicate linear staining (B) or negative staining but with diffuse weak staining in the connective tissue (C), × 100.

Mucosal hyperplasia, dysplasia, carcinoma in situ *and invasive carcinoma*

During the second week of the experiment, mucosal epithelium, although histologically normal (Fig. 2A), had mild hyalinous changes accompanied by a weak but diffuse immunoreactivity of tenascin in the submucosal connective tissue (Fig. 2B). During the third to fourth week of the experiment, a slight hyperplasia of the epithelial layer (Fig.

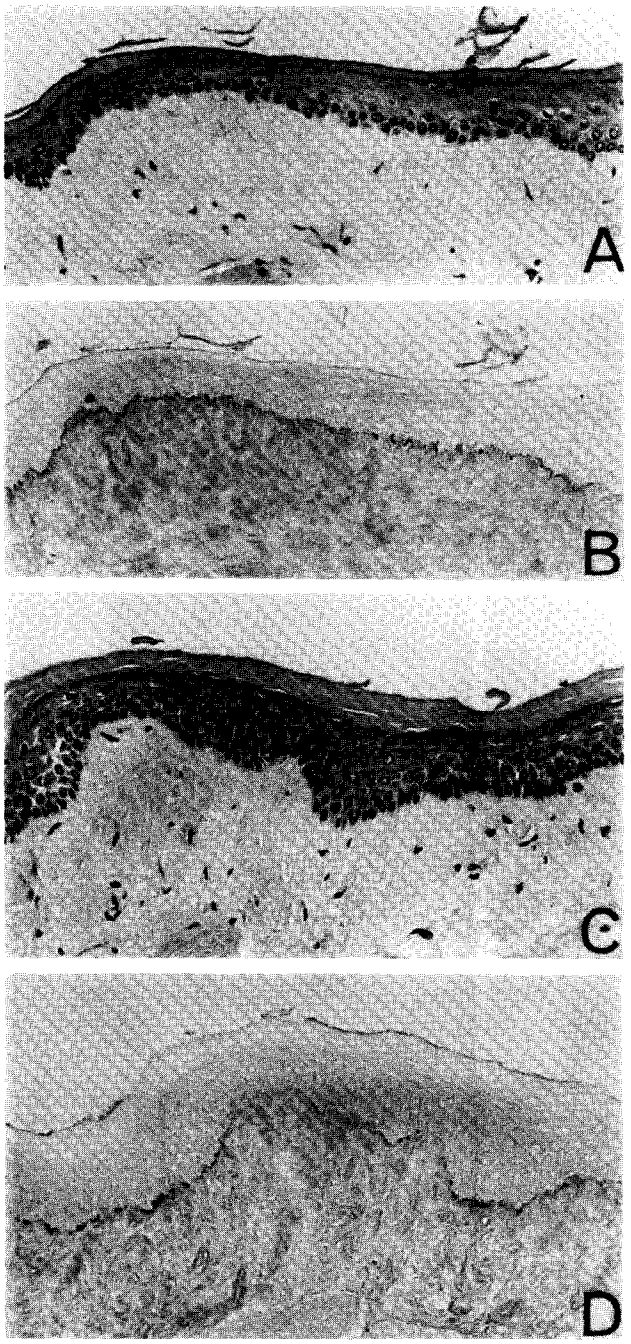
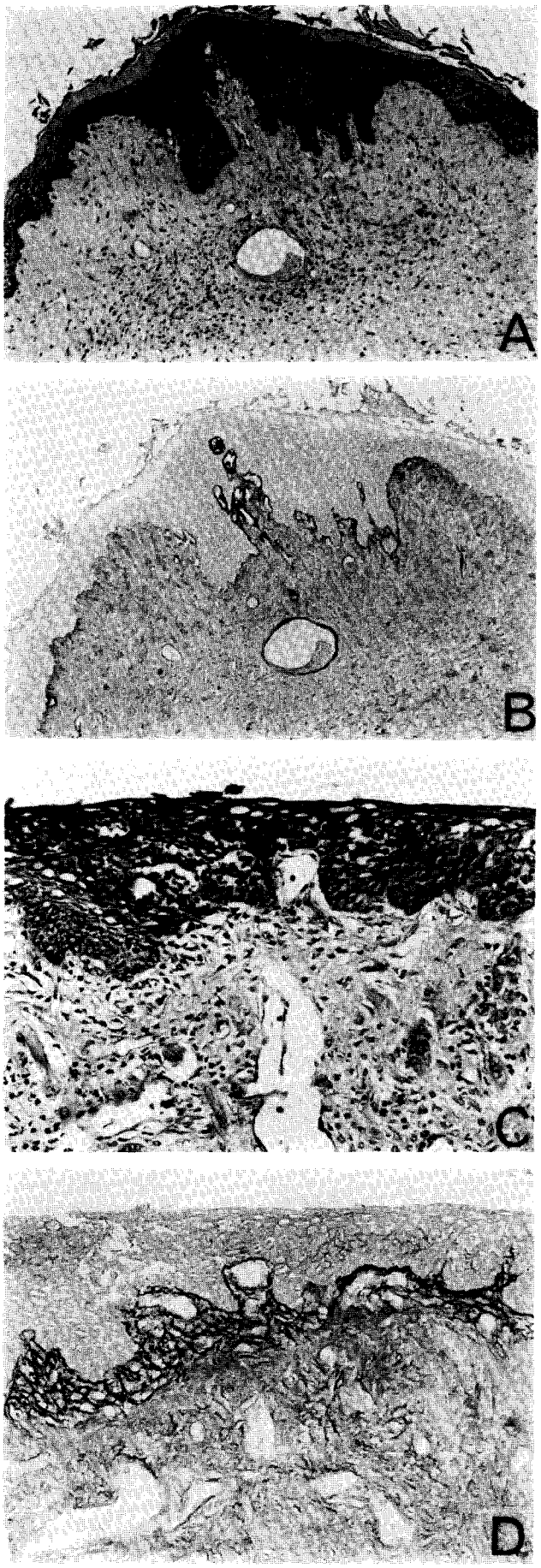


Fig. 2. Haematoxylin and eosin stained sections following second (A) and fourth (C) week of DMBA application show hyalinous changes in the submucosal connective tissue. Basal cells of the epithelium are proliferating (C). Tenascin immunostaining is weak but diffusely positive in the submucosal connective tissue, with condensed staining at the epithelial–connective tissue interface ((B) and (D)).



2C) had positive immunoreactivity condensed at the sites of epithelial down growths and the weak but diffuse reactivity at the subepithelial connective tissue persisted (Fig. 2D). During the fifth week of the experiment, the hamster buccal pouch mucosa showed hyperkeratinisation and short rete pegs with multiple focal basal cell hyperplasia (Fig. 3A) and the mesenchyme at the immediate vicinity of the hyperplastic epithelium, particularly in between the rete pegs had an intense immunoreactivity of tenascin (Fig. 3B). The sixth week specimens showed progressive hyperplasia with mild dysplasia and the immunoreactivity of tenascin was more widespread along the epithelial–mesenchymal interface. The specimens after 8 weeks showed advanced epithelial dysplasia accompanied by carcinomatous changes (Fig. 3C) and had a more widespread immunoreactivity in the mesenchymal tissue adjacent to the lesional epithelium (Fig. 3D). Ulcerations on the buccal pouch mucosa were observed after the ninth week and the early invasive carcinoma cells were surrounded by stromal tissues intensely reactive for tenascin. However, the connective tissue at distant sites was negative (Fig. 4A, B). As the carcinoma advanced with invasive tumour cells extending deep into the connective tissue, often with multiple nodules and necrotic changes in the tumour mass, widespread stromal immunoreactivity of tenascin was observed (Fig. 4C, D). Under high power, immunoreactivity was also observed intracellularly and on the cell surface of tumour cells (Fig. 4E). Immunoreactive tenascin was widespread throughout the tumour stroma in well-advanced invasive carcinoma.

DISCUSSION

Hamster buccal pouch mucosa following topical administration of DMBA is unique in its fidelity with respect to histomorphological and functional aspects of human oral leukoplakia and squamous cell carcinoma. As in animal models of neoplasia and human neoplasia by virtue of itself, cellular events leading to tumorigenesis and tumour progression with invasive neoplastic lesions at the molecular level are yet to be fully investigated. However, to have appropriate model systems for basic research, animal models for human neoplasia should approximate the human conditions to an extent relevant to the morphological and biological behaviour. The diverse events at the molecular level leading to human neoplasia may not necessarily be the same in an animal model. However, it is necessary to investigate how closely the DMBA-induced carcinogenesis in hamster buccal pouch mucosa mirrors human oral carcinogenesis at various levels and does this system have any relevance other than as a model.

In the present study, following application of DMBA, the hamster buccal pouch mucosa showed hyperkeratinisation in the fifth week, epithelial dysplasia in the sixth and seventh week, and carcinoma *in situ* with features of early invasive carcinomas were observed in the eighth week in all experimental animals. Subsequently, there were ulceration with

Fig. 3. Epithelial–connective tissue interface in areas of hyperplastic epithelium (fifth week specimen) focally show positive tenascin immunostaining particularly between the rete pegs ((A) HE stained section; (B) tenascin immunostaining), × 100. Tenascin immunostaining is more widespread in the mesenchyme in the vicinity of carcinomatous area (ninth week specimen, (C) HE stained sections, (D) tenascin immunostaining), × 100.

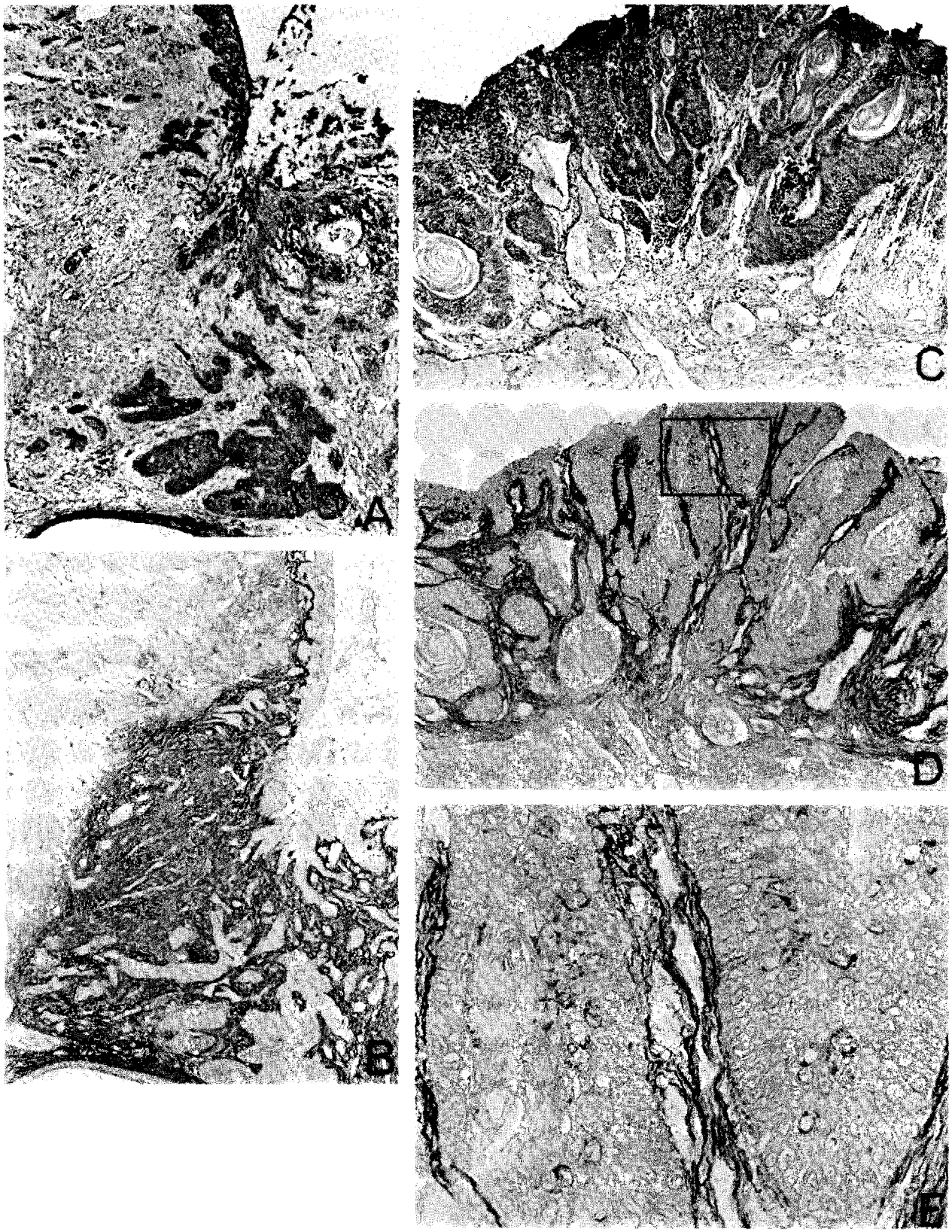


Fig. 4. A widespread *tenascin immunostaining* is seen in the stromal tissues of invasive carcinoma with distant connective tissue showing negative staining (11th week specimen, (A) HE staining, (B) tenascin immunostaining), ×40. The 13th week specimen shows a widespread interstitial and peripheral connective tissue stroma positive for tenascin ((C) HE staining; (D) tenascin immunostaining), ×40. (E) High power view of inset within (D), reaction products are also localised within the cytoplasm and cell surface of numerous tumour cells (×200), the significance of which is unknown.

formation of cancer nodules, often multiple, as the lesion advances and by the 13th week there were areas of extensive necrosis with infiltrating growth within the tumour mass. It is often difficult to ascertain the sequence of cellular events in humans, and oral mucosal cancer, in a significant number of cases, is not always preceded by an obvious preneoplastic lesion and a precancerous lesion or condition, even after a prolonged period of time, does not always progess into a malignant lesion. However, the induced carcinogenesis in an animal model should mirror the events in human neoplasia at least to some extent at the molecular level.

The results of the present study showed that, in terms of expression of tenascin, during the sequential events of DMBA-induced carcinogenesis in hamster buccal pouch mucosa, the stromal immunoreactivity positively correlates with the advancing lesion. The hyalinous changes in the subepithelial connective tissue before any obvious changes in the epithelium may be an altered epithelial–connective tissue interaction during the very early stages of DMBA application. Tenascin alters the cell–cell and cell–extracellular matrix interaction and may have a role in facilitating the tumour invasion and metastasis. A number of human neoplasia show an enhanced stromal immunoreactivity of tenascin [12–19] and in oral leukoplakia the enhanced expression has been found to be related to the degree of epithelial hyperplasia and/or dysplasia and, in oral carcinomas, it may have an important role during active phases of tumour cell proliferation and stromal changes [11].

The animal model and the immunohistochemistry in the present study, as in the previous studies of human neoplasia, fail to demonstrate the cells responsible for enhanced expression of tenascin *in vivo*. Intracellular and cell surface positive immunoreactivity on the epithelial tumour cells in the present study, if it signifies production and secretion of tenascin, needs further verification by studies such as *in situ* hybridisation at the level of mRNA or cDNA to elucidate the role of epithelial cells for stromal immunoreactivity of tenascin. In *in vitro* experiments, tenascin has been found to be produced by a number of human carcinoma cell lines [20]. However, so far experimental evidence still suggests the role of mesenchymal tissues in its production [21]. In this context, experimental carcinogenesis in an animal model, as seen in the present study, would be an appropriate experimental design to study the modulation of tenascin in neoplastic lesions and to define its biological roles.

At the molecular level, tenascin shows structures homologous to epidermal growth factor and purified tenascin has been found to stimulate proliferation of various carcinoma cells suggesting a growth factor-like function [8]. In addition, growth factors produced by the tumour cells, such as transforming growth factor beta stimulate the production of tenascin by mesenchymal cells and in turn it may stimulate tumour growth, setting up a vicious cycle [22]. Moreover, tenascin may share some of the integrin receptors and affect the cell–cell or cell–extracellular interaction as it has been found to interact with heparan sulphate, a component of the basement membrane, and interfere with the cell binding function of fibronectin [6, 7]. With this multifunctional perspective, tenascin in human neoplasia, and its expression in experimental carcinogenesis of hamster buccal pouch mucosa as an animal model, has an implication in the study of neoplastic lesions at one of the molecular levels. The results of the present study suggest that expression of tenascin in experimental carcinogenesis of hamster buccal pouch mucosa, as a model, faithfully mimics the same in human oral mucosa.

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