Immunohistochemical Expression of Fibroblast Marker TE-7 in Human Dental Pulp Cells

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ABSTRACT

Introduction: Fibroblasts are currently identified by exclusion, as no specific fibroblast marker is presently available. Positive identification of these cells therefore remains challenging. The anti-fibroblast antibody, TE-7 clone was proposed as a fibroblast-specific marker. This research aimed to evaluate the application of the anti-human anti-fibroblast TE-7 clone expression in fibroblasts of the human dental pulp as an aid in identification of these cells.

Method: Twenty-four routinely extracted human teeth were collected. The dental tissues were fixed, decalcified, routinely processed and embedded in paraffin wax for Haematoxylin and Eosin staining and immunohistochemical analysis. Demonstration of monoclonal mouse anti-human anti-fibroblast antibody, TE-7 clone expression was performed on 3μm formalin fixed paraffin embedded tissue sections. Detection of the antibody binding was performed using the Novolink™ Polymer Detection System.

Results: Positive anti-fibroblast antibody, TE-7 clone staining was observed in fibroblasts and the collagenous stroma of the dental pulp. The odontoblasts lining the pulp periphery were negative with this clone, while the pulpal blood vessel walls revealed distinct staining.

Conclusion: Although the anti-fibroblast antibody, TE-7 clone is a sensitive marker for the identification of dental pulp fibroblasts, it is not specific in their identification. This particular clone may be useful as a marker for pericytes of the blood vessel walls of the dental pulp vessels.

KEYWORDS: fibroblasts, TE-7, dental pulp, pericytes

INTRODUCTION

The dental pulp is a unique environment comprised of soft connective tissues and cells enclosed by mineralised dentine. Cell types present in the dental pulp include dental pulp stem cells, macrophages, odontoblasts and immune cells. However, fibroblasts form the largest cell population in the dental pulp.1,2 Besides the fibroblasts’ known function in maintaining the integrity of the surrounding connective tissue through collagen synthesis and degradation, these cells also play an important role in wound healing.3 In the dental pulp, fibroblasts have been implicated in the tooth’s response to caries or trauma. Furthermore, fibroblasts have been proposed as a possible origin of odontoblast-like cells involved in these reactions.4

Histological identification of fibroblasts is generally based on their spindle/stellate shape. This is combined with positivity or negativity for specific immunohistochemical markers (positivity for vimentin and collagen 1α1, and negativity for epithelial, muscle, astrocyte and hematopoietic cell markers). Additionally, fibroblasts from different tissues may have differing morphological appearances, and depending on the cells current function, can have different shapes.5-10 A number of antibodies [(vimentin, anti-fibroblast surface protein (1B10), fibroblast marker antibody (ER-TR7), heat shock protein 47 (HSP47), procollagen type 1, anti-fibroblast growth factor receptor 4 (FGFR4) antibody (clone 5B5)] are reported to identify fibroblasts. However, none of these markers is exclusive in the identification of fibroblasts and staining remains non-specific.11-17

In 2008, an anti-fibroblast antibody; TE-7 clone was proposed as a specific and sensitive marker for the identification of fibroblasts.12 Subsequently, it has been used
for this purpose in various studies. To our knowledge however, the TE-7 clone has not yet been tested on dental pulp fibroblasts. Therefore, the aim of this study was to investigate the immunohistochemical expression of the TE-7 clone on human dental pulp tissue.

MATERIALS AND METHODS
Specimen Preparation
This study was carried out with the approval of the Ethics Committee of the Faculty of Health Sciences, University of Pretoria (215/2018).

Teeth undergoing routine extraction were collected and placed in 10% neutral buffered formalin for fixation of the pulpal tissues. The root apices were cut off (approximately 1/3rd of the root) using a diamond cut-off wheel (Struers BOD15) under running water, allowing the formalin to adequately penetrate the root. Once again, the teeth were placed in formalin for 48 hours. After the fixation period, the teeth were decalcified in a 10% (w/v) ethylenediaminetetraacetic acid/phosphate buffered saline solution pH 7.4 for 22-28 weeks. Adequate decalcification of the teeth was determined by cutting the tooth with a scalpel, without any resistance. The two bisected halves, longitudinally sectioned, were routinely processed and embedded in paraffin wax blocks and labelled numerically as portions 1A and 1B of tooth number 1 respectively. Optimal fixation was morphologically confirmed on Haematoxylin and Eosin stained sections.

Immunohistochemical analysis
Immunohistochemical staining to demonstrate TE-7 clone expression was performed on 3μm formalin fixed paraffin embedded (FFPE) tissue sections. The sections were baked at 60°C overnight, dewaxed in xylene and hydrated through a graded series of alcohol to distilled water. Heat induced epitope retrieval was done in a water bath at 62°C for 90 minutes in low pH (citrate based) retrieval solution (Ventana CC2 material number 05279798001, Roche Diagnostics, Ventana Medical Systems, Inc, Tucson, Arizona). This was followed by two changes of distilled water rinses for 5 minutes each. All rinse steps thereafter were performed in two changes of fresh 0.01M phosphate buffered saline (PBS)/0.5% Tween 20 pH 7.4 at room temperature. Sections were treated with a 3% aqueous hydrogen peroxide solution for 10 minutes at room temperature to quench endogenous peroxidise. This was followed by rinsing. To reduce non-specific binding of antibodies (primary and polymer) to tissue protein and Fc receptors, sections were incubated in Protein Block (Novolink Polymer Detection System, RE7280-K, Leica Biosystems Newcastle Ltd, Newcastle Upon Tyne, UK) for 5 minutes. After rinsing, the sections were incubated overnight at 4°C with a 1:10 dilution of monoclonal mouse anti-human anti-fibroblast antibody, clone TE-7 (Labome CBL271, EMD Millipore, Merck KGaA) using Novocastra™ IHC Diluent (RE7133, Leica Biosystems Newcastle Ltd). This was followed by rinsing. Detection of antibody binding was performed using the Novolink™ Polymer Detection Kit (Novolink™ RE7280-K, Leica Biosystems Newcastle Ltd).

Sections were incubated in Post Primary solution for 35 minutes. This was followed by rinsing of the slides and incubation in the Novolink™ Polymer for 35 minutes. Sections were rinsed before incubation with a 1/20 working solution of 3’, 3’diaminobenzidine (DAB) chromogen in DAB substrate buffer (Novolink™ Polymer Kit) for 5 minutes to demonstrate horseradish peroxidase (HRP) labelling. The sections were then rinsed again. The sections were then counterstained in Haematoxylin (Shandon™ Instant Hematoxylin, Thermo Scientific™ cat no 6765015) for one minute and blued in tap water. The slides were dehydrated in a series of alcohol solutions, cleared in xylene and mounted with DPX™ permanent mounting medium.

Thymus²⁴ and fibrous epulis tissue served as positive controls and the absence of staining in cells that did not express TE-7, were used as internal negative controls. An additional negative control was performed on dental pulp tissue sections substituting the primary antibody with PBS/Tween solution. A fibrous epulis is a gingival growth characterised by an increase in fibroblasts and fibrous tissue as a result of chronic irritation.²⁵ Immunohistochemistry, was performed on both decalcified and non-decalcified fibrous epulis tissue to determine if the decalcification process had any influence on the TE-7 clone.

All photomicrographs were taken with a Nikon DS-Ri1 camera mounted on a Nikon Eclipse E1000 microscope using NIS-Elements BR 4.50 software.

RESULTS
Twenty-four teeth with adequate fixation and decalcification were prepared for immunohistochemical analysis. As confirmed by the controls, TE-7 clone expression was not altered by the decalcification process. Only one section (A or B) of each tooth was included as part of the study.

All twenty-four sections showed a similar staining pattern. Positive staining for the TE-7 clone was observed in fibroblasts and fibrous tissue as a result of chronic irritation.²⁵ Immunohistochemistry was performed on both decalcified and non-decalcified fibrous epulis tissue to determine if the decalcification process had any influence on the TE-7 clone.

TE-7 clone was proposed in 2008 as a specific and sensitive marker for the identification of fibroblast

Figure 1. Overview of immunohistochemical staining of the TE-7 clone in the human dental pulp (Magnification × 40)
A fibrous epulis is a gingival growth characterised by an increase in fibroblasts and fibrous tissue

The fibroblasts demonstrated in the stained sections of this study were identified based on their histological appearance of being stellate/spindle shaped, as well as the absence of α-SMA expression. The odontoblasts lining the pulp periphery were negative with this clone (Figure 2).

An interesting finding was that the blood vessel walls displayed distinct staining using this particular clone (Figure 2). On closer examination, in conjunction with other immunohistochemical markers (α-SMA and CD34), pericytes were found to be positive with this clone (TE-7) while the endothelial cells were not (Figure 3 and Figure 4).

DISCUSSION

The anti-fibroblast antibody, TE-7 clone was first used by Haynes to examine the human thymic environment.24 Within that context, staining defined the mesodermal component of the thymus, specifically, the thymic capsule, fibrous tissue and vessels in the interlobular septae. The antibody also reacted with the cartilage, vessels and mesenchymal interstitial cells in early foetal tissues. Additionally, a number of normal tissues, malignant tumour cells and cell lines were tested using that particular clone. Of all the normal tissues tested those authors demonstrated TE-7 positivity in the fibrous stroma and blood vessels. Of the malignancies tested, only the fibrosarcoma showed positivity with that clone.24

Anti-fibroblast antibody, TE-7 was later used for the identification of fibroblasts in FFPE tissues. Cultured fibroblasts from normal lung and dermis (foreskin) were tested with various fibroblast antibodies (clones TE-7, 1B10 and 5B5) and control antibodies (SMA, CD68 and vimentin). The results showed that 50% of the lung fibroblasts and 50% of the growing and quiescent dermal fibroblasts were positive when using the TE-7 clone.12 Further testing of TE-7 reactivity on a variety of other tissues found the TE-7 clone to show stronger and more specific staining of fibroblasts when compared with the 5B5 and 1B10 antibody clones. The spindle-shaped cells in the adventitia of the large blood vessels and regions surrounding small blood vessels throughout the skeletal muscle area were also positive when using the TE-7 clone.12 However, TE-7 did not routinely stain smooth muscle cells surrounding the vessels and those authors considered TE-7 to recognise a protein of the endomysium that surrounds the muscle fibres.12 Pilling and colleagues22 used the TE-7 clone to distinguish monocyte-derived fibrocytes from monocytes, macrophages and fibroblasts. They found foetal fibroblasts and cultured normal human dermal fibroblasts to be positive when using this clone. Whereas fibrocytes and macrophages were negative.22 A study on the differentiation of skeletal muscle fibroblasts from adipose cells further supported that the TE-7 clone appears to be the most discriminating marker for fibroblasts.11

Fibroblasts are involved in maintenance of the dental pulp and have been implicated in the tooth’s response to injury.4 The odontoblasts lining the periphery of the dental pulp have the same origin as the pulpal fibroblasts, differentiating from ectomesenchymal cells of the dental papilla.26 Additionally, pulp fibroblasts have been suggested to serve as a possible source of odontoblast-like cells during response to pulpal injury.27-29 However, in this study, the odontoblasts lining the pulp periphery as well as the odontoblast-like cells found in areas of previous injury were negative when using the TE-7 clone.

In all tooth sections, distinct positivity with the TE-7 clone was observed around the blood vessels of the dental pulp. In 1926, Wellings stated that the walls of the blood vessels of the tooth pulp were very delicate and that it was not easy to observe their structure.28 More recently, the blood vessels of the tooth pulp were described to be composed of endothelial cells surrounded by a continuous layer of flat pericytes.8 Current dogma accepts that pericytes reside within micro-vessels while smooth muscle cells surround the vascular wall of larger
CONCLUSION
The results of this study show that anti-fibroblast antibody, TE-7 clone is a sensitive marker for the staining of pulp fibroblasts. However, it is not specific for pulp fibroblasts and therefore cannot be classified as a fibroblast specific marker in the context of the human dental pulp. Results indicate that this clone maybe useful as a marker for pericytes of the blood vessel walls of the dental pulp vessels.

Conflict of interest
The authors declare that there was no conflict of interest.

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