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Angiogenesis in the reparatory mucosa of the mandibular edentulous ridge is driven by endothelial tip cells

RUXANDRA STĂNESCU[1)], ANDREEA CRISTIANA DIDILESCU[2)], ADELINA MARIA JIANU[3)], M. C. RUSU[4)]

[1)]*Discipline of Oral Implantology*
[2)]*Discipline of Embryology*
Faculty of Dental Medicine,
"Carol Davila" University of Medicine and Pharmacy, Bucharest
[3)]*Department of Anatomy and Embryology,*
"Victor Babeş" University of Medicine and Pharmacy, Timisoara
[4)]*Discipline of Anatomy,*
Faculty of Dental Medicine,
"Carol Davila" University of Medicine and Pharmacy, Bucharest

Abstract

Sprouting angiogenesis is led by specialized cell – the endothelial tip cells (ETCs) which can be targeted by pro- or anti-angiogenic therapies. We aimed to perform a qualitative study in order to assess the guidance by tip cells of the endothelial sprouts in the repairing mucosa of the edentulous mandibular crest. Mucosa of the mandibular edentulous ridge was collected from six adult patients, prior to healing abutment placement (second surgery). Slides were prepared and immunostained with antibodies for CD34 and Ki67. The abundant vasculature of the lamina propria was observed on slides and the CD34 antibodies labeled endothelial tip cells in various stages of the endothelial sprouts. Ki67 identified positive endothelial cells, confirming the proliferative status of the microvascular bed. According to the results, the *in situ* sprouting angiogenesis is driven by tip cells in the oral mucosa of the edentulous ridge and these cells can be targeted by various therapies, as required by the local pathologic or therapeutic conditions.

Keywords: CD34, Ki67, oral mucosa, edentulous mandibular ridge.

Introduction

Angiogenesis is the process that generates new blood vessels and capillaries from pre-existing blood vessels. This process initially involves proliferation, sprouting, and migration of endothelial cells [1, 2]. The newly generated blood vessel sprout is guided by migrating tip cells [1].

The distal end of each sprout contains a specialized endothelial cell (EC), termed tip cell, which is motile, invasive and dynamically extends long filopodial protrusions; the base of the endothelial sprout is formed by additional ECs, termed stalk cells [2]. Few vascular sprouts do extend significantly beyond a distance of 100 μm before they form new connections. In order to expand the vasculature over larger distances, repetitive steps of endothelial sprouting and tubulogenesis are required [2].

Consequently, the current model of endothelial angiogenesis centers on the interplay between "tip" and "stalk" cell characters [3, 4], and Notch signaling is central to the establishment of these identities [3]. Tip cells comprise so a distinct subpopulation of endothelial cells which constitute an attractive target for pro- and anti-angiogenic therapy [5].

CD34 is expressed in endothelial cell filopodia at sites of active angiogenesis *in vivo*. Also, the tip cell phenotype and CD34 expression co-occur in endothelial monolayers *in vitro* [5].

The microvessel density was found to be significantly increased in a relatively large spectrum of pre-malignant squamous cell lesions, such as in the oral mucosa or in skin [6].

It was also suggested that mechanical compression of the palatal plate induces ischemia, and that cells in the underlying denture-supporting tissue, which includes the periosteum, synthesize VEGF to maintain homeostasis under these conditions [7].

We aimed to perform a qualitative study in order to assess the guidance by tip cells of the endothelial sprouts in the repairing mucosa of the edentulous mandibular crest.

Materials and Methods

Human adult bioptic material (mucosa of the edentulous alveolar crest of the mandible) was collected from six adult patients (four females and two males; mean age 33.5 years, SD 3.21), prior to healing abutment placement (second surgery).

Three additional samples of mandibular ridge mucosa were collected from patients prior to the extractions of the third molar, and were used as control samples.

Informed consent for use of the bioptic material with research purposes was obtained from the patients.

The Bioethics Committee of the host institution approved the study.

The collected samples were fixed for 24 hours in buffered formalin (8%) and were processed with an automatic histoprocessor (Diapath, Martinengo, BG, Italy) with paraffin embedding.

Sections were cut manually at 3 µm, and were mounted on SuperFrost® electrostatic slides for immunohistochemistry (Thermo Scientific, Menzel-Gläser, Braunschweig, Germany).

Histological evaluations used 3 µm thick sections stained with Hematoxylin and Eosin.

Anti-CD34 (clone QBEnd 10, DAko, Glostrup Denmark, 1:50) and anti-Ki67 primary antibodies (clone MIB-1, Dako, Glostrup Denmark, 1:50) were used.

Sections were deparaffinized, rehydrated and rinsed in phosphate-buffered solution (PBS) at pH 7.4.

Retrieval by cooking in specific buffer was completed: (a) for CD34: EDTA (pH 9, 20 minutes); (b) for Ki67: 0.01 M citrate retrieval solution (pH 6, 20 minutes).

Appropriate endogenous blocking peroxidase was completed before immunolabeling (0.1% BSA in PBS).

Sections incubated with non-immune serum served as negative controls.

Sections were counterstained with Hematoxylin.

Normal colon samples, bioptic, were used as external positive controls for both antibodies.

The microscopic slides were analyzed and micrographs were taken and scaled using a Zeiss working station: AxioImager M1 microscope with an AxioCam HRc camera and AxioVision digital image processing software (Carl Zeiss, Oberkochen, Germany).

Results

On HE stained slides the general histology of the mucosa was evaluated, the epithelium and the lamina propria were accurately identified, the later consisting of a superficial, papillary layer, and a deep, reticular layer.

On CD34 immunolabeled slides of control samples, the general pattern of the lamina propria microvasculature was evaluated. The microvessels of the reticular layer were rather parallel with the epithelial surface, and were building a well-represented reticular micro-vascular network. The papillary microvessels seemed to irradiate from the reticular layer and were coursing perpendicularly on the reticular network to penetrate the papillae between the epithelial ridges, in a *vasa recta* fashion (Figure 1).

CD34 antibodies labeled all microvessels, seemingly discontinuous, in lamina propria of the edentulous ridges, both in the papillary layer, and in the reticular layer (Figures 2 and 3).

Beyond the remarkable microvascular density that was observed on all slides, CD34 accurately identified the endothelial tip cells and the particular appearance of their filopodial sprouts (Figures 2 and 3).

Different stages, of sprouts initiation, extension, bridging and lumenization of the newly formed tubes (Figure 3) were evidenced.

Ki67 positively labeled the basal cells of the epithelium and also identified positive endothelial cells (Figure 4). So, the proliferative status of the micro-vascular bed was confirmed.

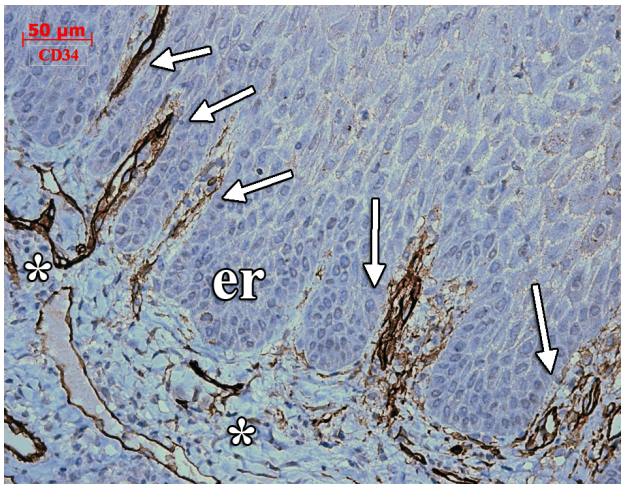

Figure 1 – ***Oral mucosa (resident, non-reparatory) of the edentulous mandibular ridge. CD34-positive microvessels build a network (*) of the reticular layer of lamina propria. Papillary microvessels (arrows) irradiate from the reticular network and penetrate between the epithelial ridges (er). Scale bar: 50 µm.***

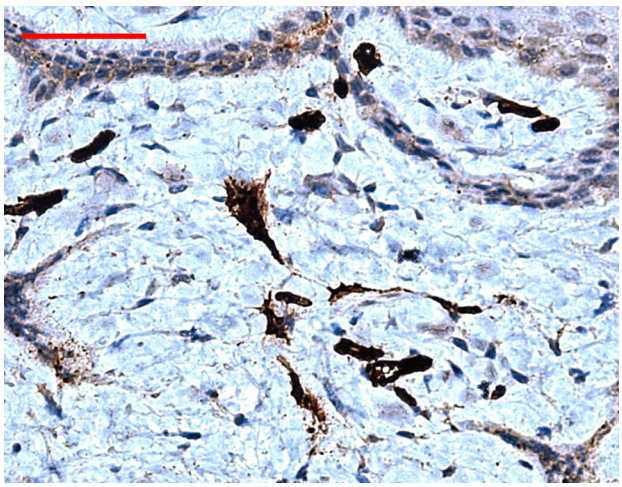

Figure 2 – ***Oral mucosa of the mandibular edentulous ridge. Abundant CD34-positive microvessels are identified in the lamina propria. Scale bar: 50 µm.***

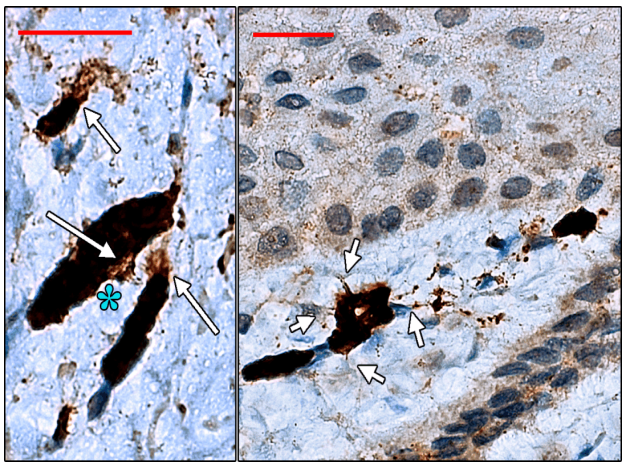

Figure 3 – ***Oral mucosa of the mandibular edentulous ridge. CD34-positive microvessels display cells with filopodial projections (arrows). (*): sprouts bridging, lumenization of a new endothelial tube, and new sprouting. Scale bars: 20 µm.***

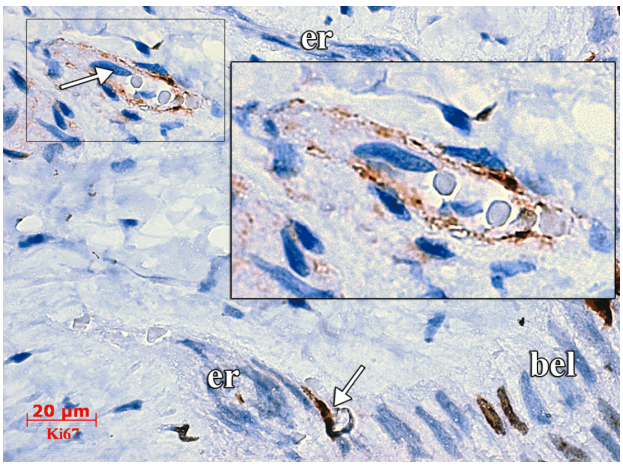

Figure 4 – ***Ki67-positive labeling of the endothelial cells of microvessels (arrows) in the papillary layer of the lamina propria (inset: higher magnification). er: epithelial ridges; bel: basal epithelial layer.***

Discussion

Blood vessel development and network patterning are controlled by several signaling molecules, including VEGF, FGF, TGF-β, Ang-1,2. The roles of VEGF signaling in endothelial cell proliferation, migration, survival, vascular permeability and induction of tip cell filopodia have been reported [8, 9].

It has been recently shown that activation of plexin D1 by Sema3E causes the rapid disassembly of integrin-mediated adhesive structures, thereby inhibiting endothelial cell adhesion to the extracellular matrix (ECM) and causing the retraction of filopodia in endothelial tip cells. A molecular framework for anti-angiogenesis signaling has been provided, thus impinging on a myriad of pathological conditions characterized by aberrant increase in neovessel formation [10].

As angiogenesis may be an early step in oral tumorigenesis [11] the specific tip cell driven mechanism of oral mucosa angiogenesis assessment is important to be known and marked as a target of antiangiogenic therapies.

Serial changes in the microvascular pattern beneath the inner gingival epithelium were studied experimentally to elucidate the process of reconstructing the vascular architecture following mucoperiosteal flap surgery [12]. In early stages the resident subepithelial capillaries were transformed in glomeruli and then new vessels were formed by sprouting angiogenesis; finally, a new subepithelial capillary network resulted accompanying the epithelization [12]. However, at that time the distinction of tip, and stalk endothelial cells was not available to correlate the results of the experiments.

It has been suggested that the presence of VEGF in salivary glands and saliva may facilitate the high healing capacity shown by oral tissues [13, 14]; however, the direct correlation between VEGF and angiogenesis in the oral mucosa needs further investigations [15].

Conclusions

In situ angiogenesis is driven by tip cells in the mucosa of the edentulous crest and these cells can be targeted by pro- or anti-angiogenic therapies, as required by the local pathologic or therapeutic conditions.

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Corresponding author
Mugurel Constantin Rusu, Senior Lecturer, MD, PhD, Discipline of Anatomy, Faculty of Dental Medicine, "Carol Davila" University of Medicine and Pharmacy, 8 Eroilor Sanitari Avenue, 050474 Bucharest, Romania; Phone +40722–363 705, e-mail: anatomon@gmail.com

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