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# Inhibition of autophagy after perforator flap surgery increases flap survival and angiogenesis

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ABSTRACT

*Background:* The survival ratio of multiterritory perforator flap is variable. Therefore, surviving mechanisms are increasingly explored to identify novel therapeutics. The condition of the choke zone is essential for perforator flap survival. In this study, we investigated autophagy in the choke zone after flap surgery.

*Materials and methods:* The flap model involved a perforator flap with three territories that was located on the right dorsal side of a rat. A total of 36 rats were divided into six groups, including the control, 0 d postoperative (PO), 1, 3, 5, and 7 d PO groups. In addition, 72 rats were divided into three groups, including a control group, a 3-methyladenine (3-MA) group, and a rapamycin group. Skin tissue of rats was used for measuring autophagy proteins, vascular endothelial growth factor (VEGF) expression, and histological examination. On day 7 after surgery, the survival ratio of each flap was determined.

*Results:* The expression of autophagy and VEGF in the second choke zone (choke II) was increased after flap surgery. Among the three groups, the survival ratio of flaps in the 3-MA group was the highest. Furthermore, the angiogenesis level in the 3-MA group in choke II was the highest among the three groups.

*Conclusions:* Autophagy was initiated by surgery in choke II, and VEGF expression in choke II was increased after flap surgery. Inhibiting autophagy after perforator flap surgery is beneficial for flap survival and for promoting angiogenesis in choke II.



## Introduction

Ever since the technique became available in the late 20th century, perforator flap surgery has been used in a variety of clinical treatments, such as large tumor removal, severe diabetic foot ulcers, and serious burns. The main goal of flap research is to obtain a larger scale flap that can easily be prepared using convenient and safe methods. In previous studies, increasing flap survival has been reported. These include using delay procedures,[1] vascular supercharging approaches,[2] and methods combined with medication.[3-5] However, these methods are not guaranteed to be safe. Therefore, natural, molecular biology-related mechanisms of flap survival have gained attention, including autophagy. During evolution, autophagy has been highly conserved. In the 1960s, de Duve invented the term "autophagy" to describe the

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degradation process of cytoplasmic constituents in single- or double-membrane vesicles.[6] The term autophagy stems from a Greek term and refers to "self-eating". In our study, we investigated the changes of inherent autophagy in perforator flaps and studied the role of autophagy after perforator flap surgery.

In the clinic, the angiosome theory is used to predict the survival area of the perforator flap.[7] Based on its angiosomes, our flap model can be divided into three territories, including the anatomical territory, dynamical territory, and potential territory (Fig. 1).[8,9] Choke vessels are potential and caliber-reduced vessel connections between two angiosomes.[9] The area between the anatomical territory and the dynamical territory is known as the first choke zone (choke I). The area between dynamical territory and potential territory is termed the second choke zone (choke II). In addition, the boundary between the survival area and necrosis is known as choke II, and the survival ratio of the potential territory is unknown.[7,10] The condition of the choke zone, including the state of choke vessels and microvessels is known to be essential for flap survival.[1,7-9,11] Several reports have shown that vessel condition was related to, for example, inflammatory activity, reactive oxygen species, and apoptosis.[4,12,13] Studies on the relationship between autophagy and flap are limited. Therefore, data on autophagy in other fields, such as cardiovascular biology, are used as a reference. The main role of autophagy is to degrade excessive proteins or organelles to maintain homeostasis for the survival of cells.[14] However, extreme autophagy activity is detrimental for cell survival.[15-18] For example, in cardiovascular diseases, autophagy is a protective factor during ischemia but is harmful during reperfusion.[16-18] There has been a heated debate on the effects of autophagy on the survival of different cells and tissues. Therefore, identifying the relationship between autophagy and flap survival is of utmost importance.

In our study, we first explored the changes of autophagy and vascular endothelial growth factor (VEGF) expression in choke zone after perforator flap surgery by measuring protein markers of autophagy and VEGF. In addition, we calculated the survival area in animal models after the use of autophagy inhibitor and activators. Finally, histological studies were performed for the determination of microvessel conditions.

## Materials and methods

### *Animals and surgical techniques*

A total of 108 adult male Sprague–Dawley rats weighing 250-300 g were obtained from the Experimental Animal Center of Wenzhou Medical University (Approval ID: SYXK-Zhe 2015-0009) (Wenzhou, China). Animals were housed in the Animal House of Wenzhou Medical University (Wenzhou, China) and were fed a standard chow and sterile water. Animal experiments were carried out in compliance with the guidelines for animal research from the National Institutes of Health and the Committee on Animal Research.

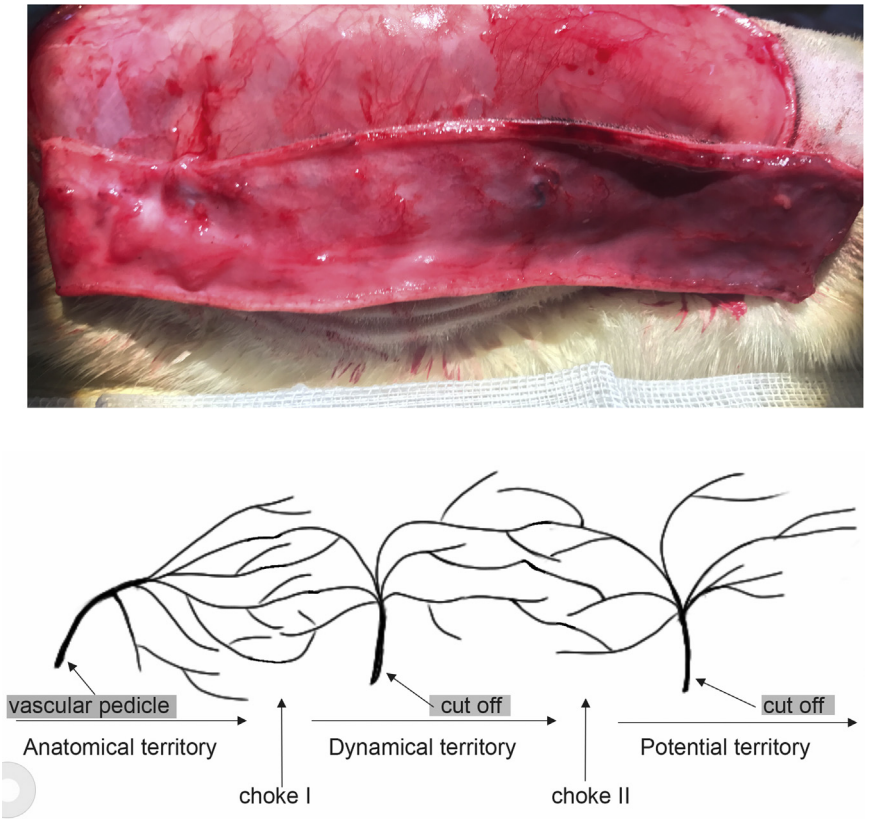


**Fig. 1 – Diagrammatic representation of angiosomes in the flap model. From the vessel pedicle to distal flap, the three territories are as follows: anatomical territory, dynamical territory, and potential territory. Choke vessels are potential and caliber-reduced vessels between two angiosomes, which are in choke zone. (Color version of figure is available online.)**