CAROLINE DE CARVALHO PICOLI

PERICYTES INDUCED BY COLD EXPOSURE IN DIFFERENT ADIPOSE TISSUES OF MICE

Instituto de Ciências Biológicas
Universidade Federal de Minas Gerais
Setembro/2022

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Tese apresentada ao Programa de Pós-Graduação em Biologia Celular do Departamento de Morfologia, do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, como requisito para obtenção do título de Doutor em Ciências.

Área de concentração: Biologia Celular

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ATA DA DEFESA DE TESE DE DOUTORADO DE CAROLINE DE CARVALHO PICOLI

Às **nove horas** do dia **05 de setembro de 2022**, reuniu-se, no Instituto de Ciências Biológicas da UFMG, a Comissão Examinadora da Tese, indicada pelo Colegiado do Programa, para julgar, em exame final, o trabalho final intitulado: **" PERICYTES INDUCED BY COLD EXPOSURE IN DIFFERENT ADIPOSE TISSUES OF MICE"**, requisito final para obtenção do grau de Doutora em Biologia Celular. Abrindo a sessão, o Presidente da Comissão, **Dr. Alexander Birbrair**, após dar a conhecer aos presentes o teor das Normas Regulamentares do Trabalho Final, passou a palavra à candidata, para apresentação de seu trabalho. Seguiu-se a arguição pelos examinadores, com a respectiva defesa da candidata. Logo após, a Comissão se reuniu, sem a presença da candidata e do público, para julgamento e expedição de resultado final. Foram atribuídas as seguintes indicações:

Prof./Pesq.	**Instituição**	**Indicação**
Dr. Alexander Birbrair	UFMG	Aprovada
Dr. Sidney Barnabé Peres	Universidade Estadual de Maringá	Aprovada
Dr. Miguel Luiz Batista Junior	UMC - Universidade de Mogi das Cruzes	Aprovada
Dra. Luciana Maria Silva	Fund. Ezequiel Dias	Aprovada
Dra. Adaliene Versiani Matos Ferreira	UFMG	Aprovada

Pelas indicações, a candidata foi considerada: Aprovada

O resultado final foi comunicado publicamente à candidata pelo Presidente da Comissão. Nada mais havendo a tratar, o Presidente encerrou a reunião e lavrou a presente ATA, que será assinada por todos os membros participantes da Comissão Examinadora. **Belo Horizonte, 05 de setembro de 2022.**

Dr. Alexander Birbrair

Dr. Sidney Barnabé Peres

Dr. Miguel Luiz Batista Junior

Drª. Luciana Maria Silva

Drª. Adaliene Versiani Matos Ferreira

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Article II: Original article:
COMPARISON OF NG2+/NESTIN+ CELLS IN ADIPOSE TISSUE FROM MICE EXPOSURE TO COLD

LIST OF ABBREVIATIONS

ATF2 = Activating Transcription Activating Factor 2

BAIBA = β-aminoisobutyric acid

BAT = Brown Adipose Tissue

BDNF= brain-derived neurotrophic factor

BMI = Body Mass Index

CD137/Tnfrs9 = tumor necrosis factor receptor superfamily member 9

CD31 = platelet endothelial cell adhesion molecule-1

CD46 = cluster of differentiation 146

CNO = clozapine-N-oxide

CREB = Cyclic AMP responsive element-binding protein 1

DPP4 = dipeptidyl peptidase-4

FDG-PET = Fluordesoxyglucose positron emission tomography and computed tomography

ICAM = intercellular adhesion molecule-1

iTAB = inguinal white adipose tissue

iWAT = inguinal white adipose tissue

iWATd = inguinal white adipose tissue distance

iWATp = inguinal white adipose tissue proximal

Metrnl = similar to meteorin 1

Myh11= myosin heavy chain 11

NG2/Cspag4 = chondroitin sulfate proteoglycan 4

PCs= pericytes

PDGF-BB = platelet-derived growth factor endothelial chemoattractant

PDGFRα = platelet-derived growth factor receptor α

PDGFRβ = platelet-derived growth factor receptor β

PGC1α = peroxisome proliferator-activated receptor-γ coactivator

PPARγ = peroxisome proliferator-activated receptor-γ coactivator

pWAT = perigonadal white adipose tissue

rpWAT = retroperitoneal white adipose tissue

RT = room temperature

Sca-1 = an 18 kDa phosphatidylinositol-anchored protein that is a member of the lymphocyte

SMA = smooth muscle

SVF = Stromal vascular fraction

T.H. = tyrosine hydroxylase

TMEM26 = transmembrane protein 26

UCP1 = uncoupling protein 1

VEGF = vascular endothelial growth factor

VEGFA = vascular endothelial growth factor A

WAT = white adipose tissue

WT = wild type

αSMA = α-smooth muscle actin

SUMMARY

GENERAL ABSTRACT

Adipose tissue remodeling, which includes phenotypic alterations and changes in vascular density, is promoted by cold exposure, which results in browning of white adipose tissue (Saito et al.), increased brown adipose tissue, and increased brown adipose tissue. Pericytes (PCs), present in the perivascular region, have been shown to be multipotent and essential for the growth and preservation of blood vessel integrity. In view of this, we aimed for this study to review the advances made in the literature to understand the plasticity of PCs in adipose tissue. To compare NG2+/Nestin+ cells in adipose tissue from mice exposed to cold. For analysis, we used NG2-DsRed/Nestin-GFP mice, which could allow the identification of pericyte subtypes. We observed the presence of two subsets of PCs in various WAT and BAT depots, the NG2+/Nestin- (type-1) and the NG2+/Nestin+ (type-2). After 2-week cold exposure at 8°C, we observed a significant increase in type 1 cells in all adipose tissues and an increase in type 2 cells in the adipose tissues most susceptible to the phenotypic changes of cold exposure. We also found that among the markers, several cells expressed only the Nestin+ marker, and that some of them appeared to be associated with blood vessels, but a small amount were not associated with blood vessels. In order to evaluate and understand if the unassociated cells could migrate to adipose tissues, we performed a parabiosis with WT and NG2-DsRed/Nestin-GFP mice and repeated the 2-week cold exposure protocol, in which we observed that there was a possible migration of NG2+/Nestin+ cells due to tissue-to-tissue contact and hypothesized that Nestin+ cells from the circulation reached more distant adipose tissues. Here, different subsets of perivascular NG2+/Nestin+ cells in adipose tissue (WAT and BAT) were revealed, which increased significantly after two weeks of cold exposure (8°C). These Perspectives discuss recent understanding of PCs in adipose tissue and their possible potential as targets for new approaches in therapeutic treatments for metabolic diseases.

Keywords: pericytes, perivascular cells; adipocytes.

1. GENERAL INTRODUCTION

Adipose tissue is considered to be an energy storage depot, however, the understanding of its role has expanded greatly. Also it is considered to be an endocrine organ, because of its ability to balance energy, secretes several proteins, adipokines, and other factors, and is related to the regulation of metabolism, immunity, and endocrine function (Fasshauer & Bluher, 2015; Galic, Oakhill, & Steinberg, 2010). Classically in the literature adipose tissue is divided into white and brown (WAT and BAT). WAT being responsible for storing energy while BAT is related to non-shivering thermogenesis (J. Wu et al., 2012) (Cinti, 2000, 2001a, 2001b).

Environmental stimuli promote phenotypic changes in adipose tissue via tissue remodeling (Bora & Majumdar, 2017; Bostrom et al., 2012; Kajimura, Spiegelman, & Seale, 2015; Xu et al., 2019). e.g. cold can activate the sympathetic nervous system, and thus, stimulate the release of norepinephrine (NE) (Virtanen et al., 2009), which binds to and activates β-adrenergic receptors (β-ARs) on adipocytes contributing to lipolysis of white and brown adipocytes, browning of WAT (Vitali et al., 2012) and increased angiogenesis (Luo et al., 2017).

It is known that vasculature represents an important source of progenitor cells, therefore, suggesting that adipogenic progenitors are part of the perivascular cells (Farrington-Rock et al., 2004; W. Tang et al., 2008) (Cai, Lin, Hauschka, & Grottkau, 2011; Lin et al., 2008; Rodeheffer, Birsoy, & Friedman, 2008; W. Tang et al., 2008). Pericytes (PCs), which are perivascular cell, could be the origin of mature adipocyte (W. Tang et al., 2008; Tran et al., 2012).

It has been shown that PCs are heterogeneous in different microenvironments and have the potential to differentiate into adipocytes, fibroblasts, muscle, vessels, and neurons (Birbrair et al., 2017), which has generated intense debate about the plasticity of PCs in various other tissues (Birbrair et al., 2017; Guimarães-Camboa et al., 2017)(Gomes et al., 2022). In the present study, we aimed to review the plasticity of pericytes in adipose tissue, we have revised the efforts made in literatures to demonstrate pericytes an adipogenic progenitors (Article I). We also compared subtypes of pericytes: NG2+/Nestin- (type-1) and NG2+/Nestin+ (type2) after 2-week cold exposure in different adipose tissue (Article II).

2. General aim

2.1. Review article aim

- Review the advances in the literature on the understanding of pericyte plasticity in adipose tissue

2.2. Original article aim

- Comparison of NG2+/NESTIN+ cells in adipose tissue in mice exposure to cold

2.2.1. Specific aims

Using white adipose tissues (iWAT, pWAT, rpWAT) and brown adipose tissues (BAT) obtained from mice exposed to 2-weeks of cold, we:

- quantify the area of adipocytes and lipid droplets (Perilipin marker);
- identify and quantify the density of blood vessels (CD31+ marker);
- identify and quantify type-1 (NG2-DsRed+/Nestin-GFP-) and type-2 (NG2-DsRed+/Nestin-GFP+) pericytes;
- evaluate other pericyte markers (PDGFRβ+) to assess if there is co-labeling with type-1 and type-2
- determine whether cold increases sympathetic tone, by quantifying tyrosine hydroxylase (T.H.) expression;
- quantify and verify whether Nestin+ cells originate from the bloodstream by performing parabiosis with Wild Type (WT) and NG2-DsRed+/Nestin-GFP+ animals

3. RESULTS

3.1. Article I: Review article

PERICYTES PLASTICITY IN THE ADIPOSE TISSUE

Caroline C. Picoli, Abdulhakeem B. Ajibike, Marisa Salvi, Bryan Ôrtero Perez Gonçalves, Gabryella S. P. Santos, Beatriz G. S. Rocha, Alinne C. Costa, Leda Maria de Castro Coimbra Campos, e Alexander Birbrair

Abstract

Several studies have suggested that extracellular matrix remodeling and the microenvironment are associated with adipose angiogenesis and adipogenesis. The dense vascular network of adipose tissue is able to supply it oxygen and necessary nutrients, and also comprise of an important niche for multipotent progenitor cells which give rise to new adipocytes that are necessary for tissue remodeling. Pericytes (PCs), which are perivascular cells, have previously been described as a potential adipogenic progenitors which can differentiate into any other cell types. This review focuses on new knowledge about the plasticity of adipose tissue PCs.

Introduction

Adipose tissue begins to develop in the mother's womb and throughout childhood (Birsoy et al., 2011; W. Tang et al., 2008). The mature white adipocytes' main roles are to store triglycerides, provide signals that control metabolism (Rosen & Spiegelman, 2006; Spiegelman & Flier, 2001), protect against trauma and cold, acting on and controlling a variety of processes, such as thermoregulation and appetite (Rousseau, Atcha, & Loudon, 2003).

Historically white adipose tissue (Saito et al.) has a defined localization, characterized by parenchymal cells containing a single large lipid droplet and sparse mitochondria, which under different physiological and pharmacological conditions can change to a more oxidative phenotype similar to brown adipose tissue (BAT), such as the presence of multilocular cells that are rich in mitochondria containing uncoupling protein 1 (UCP1) (Cinti, 2000, 2001a, 2001b). The discovery of BAT in adult humans has renewed research into WAT browning, which was first discovered in the 1990s (Cousin et al., 1992; Cypess et al., 2009; Loncar, 1991). WAT browning occurs when certain white fat pads can change into brown-like adipose tissues, during a cold exposure or β3-adrenergic agonist treatments (Kajimura et al., 2015). One of the areas in mice that is most susceptible to browning is the subcutaneous fat pad (Sharp et al., 2012; J. Wu et al., 2012) which has exceptional structural heterogeneity, and is where UCP1-expressing adipocytes develop and are referred to as inducible brown adipocytes, brite adipocytes, or beige adipocytes (Barreau, 2016 #476).

Stromal vascular fraction (SVF) niche contain cells that are extremely important in adipose tissue plasticity, angiogenesis and neovascularization (Bora & Majumdar, 2017; Klar et al., 2016). This is because, we know that the vasculature represents an important source of progenitor cells (Farrington-Rock et al., 2004; W. Tang et al., 2008), which suggests, that adipogenic progenitors are part of these perivascular cells (Cai et al., 2011; Lin et al., 2008; Rodeheffer et al., 2008; W. Tang et al., 2008). And therefore, pericytes which are perivascular cells may be involved not only with the origin of mature adipocytes (W. Tang et al., 2008; Tran et al., 2012), but could also be contributing as the emergence of beige adipocytes.

Little is known about the plasticity of PCs involved with beige adipocytes or its role in mediating angiogenesis under different condition. The main reason for this lack of knowledge is since PCs are a very heterogeneous population of cells, difficult to characterize due to the absence of specific markers. Normally the characterization of PCs

is done using several molecular markers (Morikawa et al., 2002). Although several advances have been made on various fronts regarding the origin of beige adipocytes, the topic still remains under considerable discussion. Here, in this review, we focus on new insights on the findings, which also include lineage tracing that aim to identify the origin of beige adipocytes and their cell fate, highlighting current progress in our understanding of the functions of PCs in the adipose tissue microenvironment.

PCs were first described in the 19th century by a French scientist, Charles-Marie Benjamin Rouget, who described the presence of a population of contractile cells in small blood vessels, which were designated Rouget cells (Rouget, 1873). Afterwards, these cells were renamed "pericytes" due to their distinct anatomical position around the vasculature, by a German scientist Karl Wilhelm Zimmermann (Zimmermann, 1923). And until the end of the 20th century the pericytes were identified mainly based on their anatomical location and morphology. These cells have long processes around the walls of blood vessels and are widely dispersed in all tissues (Hirschi & D'Amore, 1996). They surround the endothelial cells, and communicate with them along the length of the blood vessels by physical contact or by paracrine signaling (Díaz-Flores et al., 1991). Since adipose tissue is well vascularized, it has already been reported that there are a considerable number of PCs that are located around blood vessels (Zhang et al., 2017). Understanding the functions of PCs and the possible interactions between vascular cells and adipocytes may provide an excellent opportunity for therapeutic intervention for the treatment of metabolic diseases.

Adipose tissue

Originally, adipose tissue is considered an energy storage depot, but in recent decades, our understanding of the role of adipose tissue has greatly expanded, and because of this, this tissue has come to be considered as a major endocrine organ (Galic et al., 2010), this is due to the fact that, besides its important role in energy balance, adipose tissue can also secrete several proteins and molecules with biological activity, called adipokines, which are responsible for regulating biological processes, and can be used as markers for a variety of cardiovascular, metabolic, and inflammatory diseases (Fasshauer & Bluher, 2015).

Consensually, in the literature adipose tissue is subdivided into two classic types, white adipose tissue (Saito et al.) and brown adipose tissue (BAT). Both have phenotypic differences in their cellular composition, while also expressing a specific gene signature

(J. Wu et al., 2012). While WAT has an apparent white or yellowish color, with unilocular droplets, BAT is characterized by a more brownish appearance, with multilocular droplets, with the presence of many mitochondria that express uncoupling protein 1 (UCP1), and therefore, BAT has as its main function the burning of energy through an adaptive thermogenesis; whereas WAT presents as its main characteristic to store energy in the form of triglycerides (Cinti, 2000, 2001a, 2001b).

In addition, environmental stimuli such as cold can induce phenotypic changes. Therefore, WAT has received more attention, especially for adipocytes that are located around the vasculature, because interestingly this is where brown-like adipocytes are observed, with high expression of UCP1, as a result of adaptive thermogenesis (Jespersen et al., 2019; Kortelainen, Pelletier, Ricquier, & Bukowiecki, 1993). These white adipocytes, which express UCP1, have been a third type of adipocytes, called “beige” (Seale, 2008 #461) or "brite” (Petrovic, 2010 #460) (from “brown in white”) brown adipocytes present in the typical BAT depots are frequently referred to as "classical," "constitutive," or "developmentally programmed" brown adipocytes, whereas those present in WAT arise following permanent thermogenic induction (Giralt & Villarroya, 2013).

The microenvironment of adipose tissue can be broadly separated into mature adipocytes and stromal vascular fraction (SVF). Vascular stromal cells are a population of very heterogeneous cells, which include endothelial cells, immune system cells, fibrocytes, neurons, and adipogenic progenitors (Hwang & Kim, 2019). And although adipose tissue plays a major role in maintaining energy balance, vascular stromal cells form the niche of adipocytes and thus are able to regulate tissue function (Shamsi et al., 2021), and are therefore essential for local metabolism and homeostasis (D. C. Berry, Stenesen, Zeve, & Graff, 2013; Hepler, Vishvanath, & Gupta, 2017).

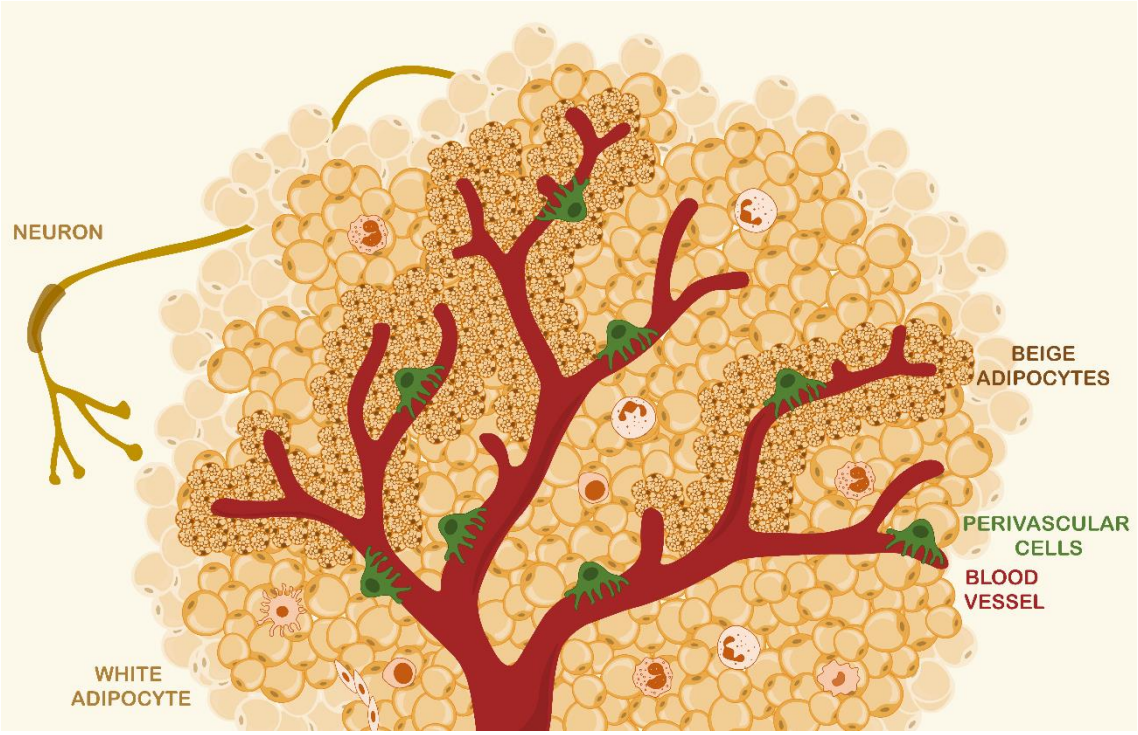


Figure 1 - Representative schematic of the white adipose tissue microenvironment, containing beige adipose cells surrounding blood vessels, neurons, perivascular cells, and other components of the stromal vascular fraction (SVF).

Adipose tissue undergoes constant remodeling processes throughout life, which is a dynamic process in response to environmental stimuli, such as temperature and/or nutritional status (Cannon & Nedergaard, 2004; Chouchani & Kajimura, 2019). However, many aspects of this remodeling still remain unclear, because it is a very heterogeneous tissue (Oguri et al., 2020).

Stem cells and adipogenic progenitors play a critical role in the remodeling of adipose tissue. Lineage tracing studies, with peroxisome proliferator-activated receptor gamma (PPARγ) demonstrated that some progenitors are PPARγ+ and that these are located on the surface of the adipose tissue vasculature, and that they also co-expressed the platelet-derived growth factor receptor β (PDGFRβ) of mural cells (pericytes) (W. Tang et al., 2008; Tran et al., 2012), suggesting, that mural cells may also represent a stem cell niche for adipose progenitors.

Vasculature in adipose tissue

Adipose tissue is embedded in a dense network of capillaries; therefore, it is highly vascularized, and the density of blood vessels is very important for the regulation of its functions and adipogenesis, and therefore, the plasticity observed in adipose tissue is highly dependent on angiogenic capabilities. Angiogenesis is a well-orchestrated process

that involves proliferation, migration, invasion, and new tube formation of endothelial cells (Herold & Kalucka, 2020).

The vascular circulatory system will be formed by an extensive network of arteries, veins, and capillaries, which are critical for the continuous delivery of supplies such as nutrients and oxygen, but also regulate the transport of adipokines, cytokines, and growth factors, remove metabolic products, and also perform paracrine regulation (Y. Cao, 2013). In addition, the vessels supply circulating stem cells, derived from bone marrow and other tissues, which are capable of differentiating into pre-adipocytes, mature adipocytes, and also into vascular cells (Y. Cao, 2010; Crossno, Majka, Grazia, Gill, & Klemm, 2006; W. Tang et al., 2008).

There are some mechanisms that angiogenic vessels also contribute to adipogenesis (Cao, 2010 #100). Further evaluating this perspective may be interesting for understanding the mechanisms that may control angiogenesis in adipose tissue, and helps to unravel potential vascular targets for possible therapeutic approaches

One of the mechanisms occurs when endothelial cells are activated in the blood vessels they can produce various growth factors and cytokines that will communicate with the adipocytes in a paracrine signaling, to promote expansion and growth (Y. Cao, 2013). Some evidence has shown that overexpression of vascular endothelial growth factor A (VEGFA) induces browning of WAT (Park et al., 2017) already, VEGFA depletion in BAT results in a whitening (Shimizu et al., 2014). Furthermore, we know that WAT and BAT are cold responsive, promoting phenotypic changes, which also occurs due to an increase of PGC-1α that will positively regulate VEGF expression and the angiogenesis process, both in *vivo* and *in vitro*, through a non-canonical pathway (Arany et al., 2008). However, this angiogenic process, triggered by cold, seems to occur temporarily, and is shown to reach a stationary level after 5 weeks in a 4°C environment (Lim et al., 2012; Xue et al., 2009).

Another interesting mechanism is that blood arteries deliver circulating stem cells that can differentiate into pre-adipocytes and vascular cells. These stem cells come from bone marrow and other organs (Crossno, 2006 #302)(Tang, 2008 #4). There are two types of bone marrow-derived stem cells that can contribute substantially to adipose tissue angiogenesis, first are circulating endothelial progenitor cells and second are multipotent mesenchymal stem cells, which can differentiate into cells and tissues of mesenchymal origin, which include adipocytes, cartilage, muscle, and bone, but which can also differentiate into endothelial cells and pericytes (Pittenger & Martin, 2004) that will

actively participate in adipose tissue angiogenesis (Y. Wu, Chen, Scott, & Tredget, 2007)(Tang, 2008 #4).

The pericytes present in the vasculature are capable of further enhancing the complex interaction between the vascular and adipose compartments. Additionally, the pericytes have the capacity to cause vasoconstriction and/or vasodilation inside the capillary beds in order to control the vascular diameter as well as the blood flow, just like the smooth muscle cells of bigger vessels. However, both cells can express contractile proteins such as α-SMA, tropomyosin and myosin, which can lead to confusion in defining the cell type. (Rucker, Wynder, & Thomas, 2000).

Pericytes also have cholinergic and adrenergic receptors (α-2 and β-2). A β-adrenergic response in the pericytes could lead to relaxation, while an α-2 response would be antagonistic and produce contraction. There are also other vasoactive substances that bind to pericytes, such as angiotensin II and endothelin 1, molecules that function as paracrine signals, which regulate pericyte contraction and relaxation, thus demonstrating that both cells interact to regulate blood flow (Rucker et al., 2000).

The pericytes that surround the capillaries have contractile proteins and are therefore considered to be ideal regulators of blood flow in the capillaries (Herman & D'Amore, 1985). Therefore, some in vitro and in vivo experiments with targeted manipulation of optogenetics for single cells have demonstrated that pericytes have the ability to control capillary diameter (Ivanova, Corona, Eleftheriou, Bianchimano, & Sagdullaev, 2021; Nelson et al., 2020; Peppiatt, Howarth, Mobbs, & Attwell, 2006; Yamanishi, Katsumura, Kobayashi, & Puro, 2006). Stimulation of the pericytes in the brain, using the ChR2 channel, responsible for causing an excitation, resulted in the contraction of blood vessels (Nelson et al., 2020), while using a Halorhodopsin channel in the retina, responsible for causing hyperpolarization, with consequent inhibition, resulting in increased blood flow in the capillaries (Ivanova et al., 2021). This ability to uniquely target the pericytes of the capillaries can be accurately and potentially important for treating circulation deficits. It remains to be understood whether single cell manipulations, using state-of-the-art technologies, including in vivo Cre/loxP mediated genetic stimulation, targeting pericytes in adipose tissue, to explore different conditions such as obesity, cachexia, lipodystrophy and others.

Adipogenic progenitors

During a process of embryogenesis the development of adipose tissue is spatially and temporally associated with the growth of microvessels (Crandall, Hausman, & Kral, 1997). Evidence suggests that capillary endothelial cells can communicate with adipocytes via paracrine signaling pathways, extracellular components, and cell-cell interactions directly (Bouloumie, Lolmede, Sengenes, Galitzky, & Lafontan, 2002; Hutley et al., 2001)

It is known that endothelial cells isolated from different adipose tissues can differ in their proliferative capacity, suggesting that adipocytes play a role in both guiding and maintaining vascular development, just as vessels also control adipocyte proliferation (Lau, 1990; Lau, Shillabeer, Wong, Tough, & Russell, 1990). Therefore, has been suggested that adipocytes and endothelial cells may share a common progenitor, which could differentiate into both adipocytes and endothelial lineages, depending on the environmental stimulus (Planat-Benard et al., 2004). Cao and colleagues observed that stem cells, from human adipose tissue, had a capacity to differentiate into endothelial cells, thus reinforcing the idea of a progenitor common to both tissues and opening possibilities for therapeutic interventions (Y. Cao et al., 2005).

The adipogenic capacity of progenitors is closely associated with metabolic changes and environmental stimuli, but there also seems to be a different capacity between different adipose, subcutaneous and visceral tissues (Hwang & Kim, 2019). In cell culture studies it was observed that subcutaneous tissue has a higher proliferative potential than visceral tissue (Rodeheffer et al., 2008; W. Tang et al., 2008), however in vivo studies using a lineage tracking system (Jeffery, Church, Holtrup, Colman, & Rodeheffer, 2015; Q. A. Wang, Tao, Gupta, & Scherer, 2013), observed that a high-fat diet can increase the proliferative state of visceral tissue, within 3 days, while subcutaneous adipose tissue did not demonstrate an increase in proliferation as quickly (Jeffery et al., 2015). These, apparent inconsistencies between the data lead us to consider that both environmental differences, the characteristic of the individual tissues, as well as the intrinsic characteristics of the adipogenic progenitors of each depot could be responsible for these inconsistencies in the literature results (Hwang & Kim, 2019).

In addition to environmental stimuli, intrinsic characteristics of the observed sample must be taken into consideration. This is because it has been shown, for example, that low temperatures can be effective in inducing the adipogenic progenitors within the WAT to form beige adipocytes, but this potential may decrease with age, appearing as a

clinical obstacle in the therapeutic use of this type of strategy in older people (D. C. Berry et al., 2017).

One of the instruments that can provide us with additional knowledge about tissue development, homeostasis, and function is the Cre/loxP site-specific recombination system (Roy, 2014 #462). Although this technique may have limitations for target mapping and progenitor identification due to the uncertainty around the timing and location of the Cre driver's actions (Magnuson, 2013 #463;Comai, 2014 #464). Modifications that enable better temporal accuracy, such as the tamoxifen-inducible Cre ER/ERT2 system (Cypess et al.) or inducible/suppressible Tet systems, have been used to solve this issue. It is assumed that only cells containing Cre can be tagged, and hence their prospective progeny, providing information regarding lineage and fate mapping. During this window, the Cre will be active together with the reporter. Therefore, scientists may identify stem/progenitor cells and outline the potential roles of these cells in development, tissue function, and homeostasis using mice that have this genetic tool (Feil, 1997 #465;Gossen, 1992 #466;Wendling, 2009 #467;Imayoshi, 2011 #468).

Berry and colleagues evaluated by lineage tracing of white adipocytes, *adiponectin*-CreER^{T2} e *aP2*-CreER^{T2}, associated with a reporter allele *Rosa26R* ^{RFP}, this white adipocytes do not generate cold-induced beige adipocytes (Berry, 2016 #160). The scientists also noted that in unilocular white adipocytes from both *adiponectin*-CreER^{T2} e *aP2*-CreER^{T2}; RFP animal models, UCP1 was not expressed. And the authors observed that amounts of adipocytes-drive-labelled, approximately 35% and -unlabelled, approximately 65%, beige adipocytes, which do express adiponectin and aP2, present after cold exposure (D. C. Berry, Jiang, & Graff, 2016). The authors also evaluated other strains and sources of mural progenitors and found that adipogenic progenitors (SMA positive) arising from mural cells also undergo differentiation into mature adipocytes, and then undergo a process of transdifferentiation, and thus present a beige adipocyte phenotype (D. C. Berry et al., 2016).

However, it is still unclear whether other progenitors contributed to the development of these initially beige adipocytes and what role other stimuli played in this phenotype. It is still necessary to elucidate whether other stimuli, such as physical training, are capable of stimulating the emergence of these beige adipocytes.

Heterogeneity of adipogenic progenitors

Adipocytes are developed during the development of mammals from mesenchyme and mesoderm (Gesta, Tseng, & Kahn, 2007) and neural crest (Fu, 2019 #469), and although bone marrow has been identified as an important source of adipogenic progenitors due to its pools enriched with mesenchymal stem cells (Sekiya, Larson, Vuoristo, Cui, & Prockop, 2004), studies that have done lineage tracing have identified that there are some genes as potential markers of these progenitors, such as platelet endothelial cell adhesion molecule-1 (CD31/PECAM-1), CD45, leukocyte antigen 76 / TER-119, platelet-derived growth factor receptor α (PDGFRα), PDGFRβ, CD24, CD34, Sca-1, dipeptidyl peptidase-4 (DPP4), and intercellular adhesion molecule-1 (ICAM-1) (Koulnis et al., 2011; Y. H. Lee, Petkova, & Granneman, 2013; Y. H. Lee, Petkova, Mottillo, & Granneman, 2012; Merrick et al., 2019; Rodeheffer et al., 2008; Schwalie et al., 2018; W. Tang et al., 2008).

Despite efforts to define a marker for adipogenic progenitors, several evidences show that surface marker genes are insufficient to localize or isolate adipogenic progenitors, because, for example, fibrotic progenitors still express adipogenic genes (Hwang & Kim, 2019; Iwayama et al., 2015; Marcelin et al., 2017; Spallanzani et al., 2019) and, moreover, these progenitors may also demonstrate different functional characteristics in many aspects, which include adipogenesis, fibrosis, vascularization, and inflammation (Burl et al., 2018; Y. Cao, 2007; Hwang et al., 2019; Merrick et al., 2019; Y. Tang et al., 2016).

Some single cell RNA sequencing analyses have demonstrated that adipogenic progenitors exhibit heterogeneous populations, in different white adipose tissues. A recent study demonstrated that in the adipose tissue microenvironment there is a hierarchy in the development of adipose progenitors that can be active during a murine adipogenesis process (Merrick et al., 2019). DPP4 positive cells (which mark multipotent progenitors) produced cells that were CD142 positive and pre-adipocytes expressing ICAM1 positive, which were ready to give rise to mature adipocytes, and therefore the authors speculate that DPP4 positive cells become committed to the adipose lineage in response to certain stimuli, thus serving as a renewable source of pre-adipocytes (Merrick et al., 2019).

Merrick and colleagues (Merrick et al., 2019) demonstrated that ICAM1 positive or CD142 positive cells could reside in the perivascular niche, and mediate adipose tissue renewal and remodeling without additional contribution from DPP4 positive cells, but it remains unclear whether these could be the only adipocyte progenitor cells during adult

animal development. However, the adipogenic potential of progenitors expressing CD142 positive is still controversial. In any case, the perivascular niche thus represents an important site where these cells reside, so that at times when homeostasis may be disturbed, they are recruited.

Pericytes

Pericytes are also known as Rouget cells or mural cells, which closely surround endothelial cells in capillaries and microvessels (Andreeva, Pugach, Gordon, & Orekhov, 1998) and are therefore predominantly in the abluminal wall of capillaries and some larger blood vessels, where they contribute to the maintenance of capillary integrity and vascular permeability (Nwadozi, Rudnicki, & Haas, 2020).

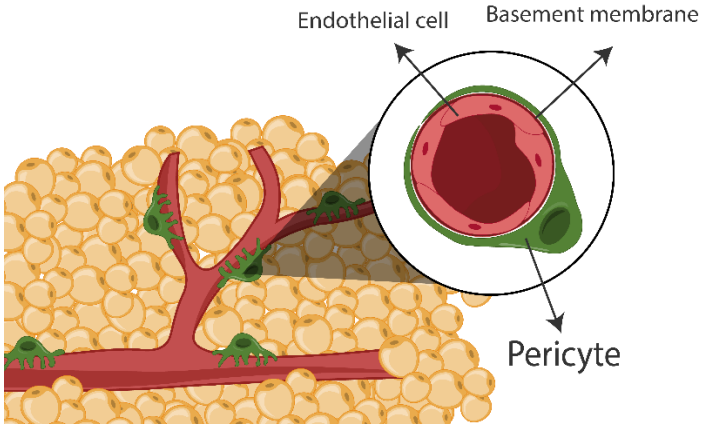


Figure 2 - Schematic representation of the location of pericytes in adipose tissue

The basal membrane of the pericyte is continuous with those of the endothelial cells and is composed of extracellular matrix proteins (predominantly collagen IV and glycoprotein laminin), which are secreted by both cell types. Morphologically, the pericytes are classified by several cytoplasmic processes emanating from a prominent cell body accompanied by several endothelial cells, and may also involve the adjacent capillaries (Bergers & Song, 2005). The pericytes emit protrusions that insert themselves into the endothelial cell invaginations (cavities) and also into occasional interruptions of the basement membrane, providing support and structure to the cell-cell communications (Armulik et al., 2005).

Usually these cells are inactive, but they can be stimulated by physiological or pathological processes, which activate vascular tissue remodeling (Birbrair et al., 2015;

Nwadozi et al., 2020). Therefore, some studies have attributed to the pericytes characteristics of a multipotent adult stem cell, since they have this capacity for self-renewal, as well as can be compromised with the cell differentiation of other lineages (Birbrair, 2019; Dore-Duffy & Cleary, 2011; Nwadozi et al., 2020).

Perivascular cells as potential progenitors of beige adipocytes

Initially it has been proposed that white adipose cells would give rise to beige adipose cells, after cold stimulus, thus occurring a process of transdifferentiation or reprogramming of adipocytes (Barbatelli et al., 2010; Smorlesi, Frontini, Giordano, & Cinti, 2012), which opened an intense debate about the origins of these cells, and it was later noted that they could be originally beige cells, with a specific gene signature and distinct origin (J. Wu et al., 2012).

Lineage tracing studies suggested that the beige adipocytes of WAT would not be associated with the proliferation of white adipocyte precursors (Y. H. Lee, Petkova, Konkar, & Granneman, 2015; Y. H. Lee et al., 2012) but with the transdifferentiation of adipocytes that already exist (Barbatelli et al., 2010; Granneman, Li, Zhu, & Lu, 2005; Himms-Hagen et al., 2000). For this reason, Wang and collaborators suggested that beige adipocytes could come from another source of progenitors (Q. A. Wang et al., 2013), since some of these beige adipocytes imply a myogenic ancestry (Kajimura et al., 2015; Sanchez-Gurmaches et al., 2012), and these progenitors that resemble smooth muscle could express Myh11, according to the group of Spielgelman (Long et al., 2014). On the other hand, Wu and collaborators observed that there is a population of CD137-positive precursors that is quiescent in WAT, and that can be activated to differentiate into beige adipocytes following an appropriate stimulus (J. Wu et al., 2012)

Some evidence suggests that vasculature is a critical component of neural, mesenchymal, and hematopoietic stem cell niches. A pioneering study by Clack and Clack (1940) had already revealed that de novo adipogenesis could occur in the close proximity of blood vessels, suggesting that adipogenic progenitors could be a type of blood vessel wall cells (endothelium, mural cells, and adventitial fibroblasts) (Cattaneo et al., 2020). Thus, some studies had proposed that perivascular cells (vascular smooth muscle cells and pericytes) could give rise not only to white adipocytes, but could potentially give rise to originally beige adipocytes and be involved in the biogenesis of these cells by de novo differentiation of adipocyte progenitor cells (Y. Jiang, Berry, Tang, & Graff, 2014; Shao et al., 2019; W. Tang et al., 2008). Hence, Berry et al. (Berry, 2016

#160) also evaluated a variety of mural cell markers (SM22, Myh11, NG2, and SMA) and found that indeed these cells would be an important source for inducing beige adipocytes, but the data obtained still need to be analyzed more extensively.

Although several efforts have been made in order to elucidate the identity of the adipogenic progenitors, there is a frequent contradiction in the results obtained in the literature, not only with lineage tracing studies, but also due to different experimental conditions (Cattaneo et al., 2020).

Pericytes, which are cells of the adventitia of blood vessels, have been implicated as a potential adult reservoir of cells that have the potential to differentiate into other cell types, such as mature adipocytes, osteoblasts, and also into other mesenchymal cell types (Corselli et al., 2012; Crisan et al., 2008; Hoshino, Chiba, Nagai, Ishii, & Ochiai, 2008; Mendez-Ferrer et al., 2010; Passman et al., 2008), becoming the object of intense investigation by some authors (D. C. Berry et al., 2017; R. Berry, Jeffery, & Rodeheffer, 2014; Gesta et al., 2007; Y. Jiang et al., 2014; Y. H. Lee et al., 2012; Long et al., 2014; Sanchez-Gurmaches & Guertin, 2014; W. Tang et al., 2008; Vishvanath et al., 2016).

In previous studies, Birbrair and colleagues identified that there are two subpopulations of pericytes, in large and small vessels, that had adipogenic, but also fibroblastic, myogenic, vascular and neuronal roles, which intensified the debate about the plasticity of these cells in various other tissues (Birbrair et al., 2017; Birbrair, Zhang, Wang, Messi, Enikolopov, et al., 2013; Birbrair et al., 2015). Therefore, to analyze more extensively the role and potential of pericytes in adipose tissue under different metabolic conditions is still necessary.

Molecular markers of pericytes and adipose tissue

To define the pericytes by a molecular marker can be a great challenge, due to the fact that no such markers have been found specifically for this cell type, and this is due to the diverse characteristics, functions and locations of the pericytes in various organs (Bergers & Song, 2005). Here, we will look at some of these markers that have been used.

During embryonic development, pericytes are normally attracted to newly formed capillaries by means of the platelet-derived growth factor endothelial chemoattractant PDGF-BB, which binds to the β receptor (PDGFRβ) (Lindahl, Johansson, Leveen, & Betsholtz, 1997), and adhesion is mediated by integrins, from the pericytes, and laminin that helps maintain PDGFRβ expression (Durbeej, 2010; Reynolds et al., 2017). Moreover, any interference with PDGF-BB / PDGFRβ signaling may be sufficient to

disrupt the interaction between pericytes and endothelial cells, indicating that this signaling is perpetuated through PDGFRβ, and is critical for maintaining pericyte localization in capillary endothelial cells (Lindahl et al., 1997).

Pericytes can also express α-smooth muscle actin (α-SMA), thereby regulating microvessel contractility (Boado & Pardridge, 1994; Nehls & Drenckhahn, 1991), but can also inhibit TGF-β activation, endothelial cell division (Betsholtz, Lindblom, & Gerhardt, 2005). Therefore, the periendothelial localization of pericytes is often confused with the localization of vascular smooth muscle cells, fibroblasts, macrophages and even epithelial cells, and although pericytes belong to the same lineage and category as vascular smooth muscle cells, it is not possible to define these cells using only a molecular marker, since they can often be confused with other cells, even mesenchymal cells (Armulik, Genove, & Betsholtz, 2011).

Therefore, it is ideal to perform a combination of general criteria that are commonly used to define this cell population, together with perivascular location and morphology. Generally, a combination of typical markers such as chondroitin sulfate proteoglycan 4 (Cspg4 / NG2), PDGFRα and PDGFRβ, differentiation cluster 146 (CD146) and Nestin (Armulik et al., 2011; Birbrair et al., 2017; Birbrair, Zhang, Wang, Messi, Enikolopov, et al., 2013; Chang et al., 2012; Dore-Duffy & Cleary, 2011; Holm, Heumann, & Augustin, 2018). And although these markers are most commonly used to characterize this population, they lack specificity because they can be expressed to some extent on other cell types, i.e., smooth muscle and interstitial cells, which can exhibit an expression pattern that is variable (Kumar et al., 2017; Sacchetti et al., 2016; van Dijk et al., 2015). Using some of these markers, some studies have investigated whether these mural cells are involved in the adipogenesis of adipocytes, white, brown, and beige.

It was shown by lineage tracing, that PDGFRα could label the progenitors of white and beige adipose tissues (D. C. Berry et al., 2016; Y. H. Lee et al., 2012; Seki et al., 2016), as well as PDGFRβ (W. Tang et al., 2008; Vishvanath et al., 2016). However, PDGFRα signaling in adipocytes seems to contribute much more to postnatal and adulthood growth, whereas PDGFRβ seems to contribute only to postnatal growth. Subsequently, in a more recent study, it was observed that PDGFRα and PDGFRβ function as inhibitors of adipogenic differentiation, this is because their deletion facilitated adipogenesis in vivo, thus suggesting that these markers may negatively regulate PDGF signaling, appearing as a critical event in the transition of adipogenic progenitors into mature adipocytes (C. Sun et al., 2020). Also, the opposite is true for

observations about PDGFRα, since PDGFRα generates fibrosis by causing adipogenic progenitors to become myofibroblast-like cells that secrete matrix proteins (Iwayama et al., 2015). Therefore, reduced signaling, or inhibition of PDGFRα could increase adipogenesis, causing myofibroblasts, thereby potentially compromising the lineage of white, brown, and beige adipogocytes (C. Sun et al., 2020).

Nestin has been used as a pericyte marker, and Nestin-GFP has served as a transgenic reporter to label subsets of cells that are pericyte-like, furthermore, these cells have demonstrated progenitor stem cell properties (Dore-Duffy, Katychev, Wang, & Van Buren, 2006; Mendez-Ferrer et al., 2010). Iwayama and colleagues, using Nestin-GFP and Nestin-Cre/TdTomato mice demonstrated that Nestin-driven transgenes are aligned with targeting perivascular cells in white adipose tissue and that these cells can contribute to young mouse adipogenesis (Iwayama et al., 2015).

Chondroitin sulfate proteoglycan 4 (Cspg4 / NG2) is expressed on the surface of pericytes during vasculogenic and angiogenic processes (Stallcup, 2002), but was also found expressed in adipocytes, demonstrating a broad activity in WAT, since genetic ablation of NG2 in mice was able to lead to obesity (Chang et al., 2012), which can impair the development of adipocytes with consequent modification of skin thickness (Kadoya, Fukushi, Matsumoto, Yamaguchi, & Stallcup, 2008).

Furthermore, it is important to point out that in other adipose tissue depots, such as the cardiac depot, NG2+ cells did not contribute to giving rise to cardiac fat. Only the PDGFRα+PDGFRβ+ cell lineage tracing of double-positive periendothelial fibroblasts contributed to the intramyocardial adipocytes (Jiang, 2021 #470).

In addition, other deposits, that were analyze using lineage tracing analyses using NG2-CreER^{T2} mice showed different results for subcutaneous and visceral adipose tissues, since the former seems to mark mature progenitor and adipocyte, requiring a cautious analysis on the formation of beige adipocytes induced by the cold of one week 6 °C, while the other tissue, visceral, showed a more specific restriction to the mural progenitor compartment, not marking mature adipocytes (D. C. Berry et al., 2016). However, more extensive analyses using the single cell technique should be done and better discussed regarding these markers, to evaluate and separate if in fact these mural cells are giving rise to adipocytes with a signature of mural progenitor markers. Furthermore, we remain unclear whether these progenitors would demonstrate the same modulation upon systematized physical training in the formation of beige adipocytes.

Environmental signals controlling the recruitment of brown and beige adipocytes

Although the functionality of BAT is still not entirely clear in adult humans, it is already well known that brown adipocytes significantly contribute to non-shivering thermogenesis by increasing heat production in animal models. Moreover, humans are also able to regulate their body temperature by behavior (clothing, heating environments, etc.) which has generated intense debate, about the functionality of this tissue (Bouillaud, Combes-George, & Ricquier, 1983; Bouillaud et al., 1988; Lean, James, Jennings, & Trayhurn, 1986) and the functional presence of BAT (Cypess et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009).

Evidence such as the presence of UCP1 in human adipose tissues since the 1980s (Bouillaud et al., 1983; Bouillaud et al., 1988; Lean et al., 1986) and also in rodents (P. Young, Arch, & Ashwell, 1984), has raised various issues regarding the physiological effects of brown adipocytes on human energy metabolism. Some observations of the presence of active BAT, in humans, (Cypess et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009), has been demonstrated by using of computed tomography and fluorodeoxyglucose positron emission tomography (FDG-PET), which is routinely used as a diagnostic tool for cancer, the ability of some glucose uptake sites that are related to BAT activity (Cypess et al., 2009; Nedergaard, Bengtsson, & Cannon, 2007; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009).

Other evidence has suggested that the amount of BAT might be negatively correlated with BMI (Body Mass Index) (Cypess et al., 2009), and be more present in women than in men (Virtanen et al., 2009). Furthermore, in biopsy samples of perithyroid adipose tissue, in which patients have undergone removal of the thyroid gland, one-third of the patients have clusters of UCP1-positive cells found within a cluster of white cells. This also correlated with a lower BMI and age of the patients. Furthermore, these UCP1 positive areas were shown to be highly innervated, demonstrating that there is a tight regulation of these cells, even in adult humans (Zingaretti et al., 2009).

Furthermore, rodent experimentation has shown that cold is an effective stimulus in the emergence of beige adipocytes in WAT, without an associated increase in the number of adipocytes, supporting the concept of transdifferentiation (Barbatelli et al., 2010). And while there is progress on the exact origin of these beige adipocyte progenitors, gaps still remain, which lend support for heterogeneity and regional specificity within each adipose tissue depot (Sanchez-Gurmaches et al., 2012).

Cold exposure has also demonstrated that the sympathetic nervous system is intimately involved in the regulation, growth and expansion of BAT and its thermogenic function, through the proliferation and differentiation of brown adipocyte precursor cells (Geloen, Collet, Guay, & Bukowiecki, 1988; Kajimura et al., 2015) (Nguyen et al., 2011; J. B. Young, Saville, Rothwell, Stock, & Landsberg, 1982), inducing the appearance of beige adipocytes within the WAT (Kajimura et al., 2015). The appearance of beige adipocytes appears totally dependent on the adipose deposit. The inguinal deposit among other deposits to be the most susceptible to phenotypic changes, while the other deposits such as periepididymal seem more resistant (Ohno, Shinoda, Spiegelman, & Kajimura, 2012; Vitali et al., 2012).

The thermogenic program also stimulates UCP1 transcription due to increased phosphorylation of transcriptional regulators, which include PGC1α, CREB, and ATF2, thus conferring a phenotypic change to the adipose tissue, which becomes more committed to shameless thermogenesis (Collins, 2011).

Although the β-adrenergic signaling pathway is a dominant circuit, some recent papers have demonstrated that there are alternative pathways that play roles in regulating beige adipocyte biogenesis (Kajimura et al., 2015), some of which do not necessarily require thermogenesis to occur, such as physical exercise (Bostrom et al., 2012; Knudsen et al., 2014; Picoli et al., 2020; Rao et al., 2014), environmental enrichment (L. Cao et al., 2011), bariatric surgery (Neinast et al., 2015; Rachid et al., 2015), cancer and cachexia (Kir et al., 2014; Leal, Lopes, Peres, & Batista, 2020; Petruzzelli et al., 2014), innate immunity (Brestoff et al., 2015; Qiu et al., 2014) local hyperthermia (Li, 2022 #473) and endocrine hormones (During et al., 2015; Ohno et al., 2012; K. Sun et al., 2012).

Among these above-mentioned stimuli, physical training is an excellent non-pharmacological strategy, important not only in the prevention, but also in the treatment of some metabolic diseases that include obesity and type 2 diabetes. And although it is well established that exercise induces adaptations to the skeletal muscle and cardiovascular system, several studies have shown that the adipose tissue contributes to the improvement of the metabolic rate as a result of profound changes in its functioning (Vidal & Stanford, 2020).

Some exercise-induced adaptations to adipose tissue include increased mitochondrial activity (Stallknecht, Vinten, Ploug, & Galbo, 1991; Sutherland, Bomhof, Capozzi, Basaraba, & Wright, 2009), decrease in cell size and lipid content (Peres, 2005 #472)(Craig, Hammons, Garthwaite, Jarett, & Holloszy, 1981), reduction of local

inflammation (Geng et al., 2019; S. Lee et al., 2019), in addition to the presence of brown adipocyte-like adipocytes within the WAT (Bostrom et al., 2012; L. Cao et al., 2011; De Matteis et al., 2013; Picoli et al., 2020; Sutherland et al., 2009; Trevellin et al., 2014).

Although the context of WAT browning may not make sense with physical training, due to the increased heat production (Saugen & Vollestad, 1995) several hypotheses have been proposed as the underlying mechanism, such as the increase in sympathetic innervation that may occur during exercise (Nedergaard & Cannon, 2014), as well as the release of myokines, such as irisin (Bostrom et al., 2012), similar to meteorin 1 (Metrnl) (Rao et al., 2014), myostatin (Feldman, Streeper, Farese, & Yamamoto, 2006), or β-aminoisobutyric acid (BAIBA) (Roberts et al., 2014) and also by some secreted factors, such as brain-derived neurotrophic factor (BDNF) (L. Cao et al., 2011).

Conclusion

PCs are cells potentially involved in the emergence of beige adipocytes in TAB. Our understanding of the role of pericytes in the various white and brown adipose tissues remains limited. Although several advances have been made on various fronts regarding the origin of beige adipocytes, this topic still remains under considerable discussion. Here, in this review, we check recent findings, which also include lineage tracing that aim to identify the origin of beige adipocytes and their cell fate, highlighting current progress in our understanding of the functions of pericytes in the adipose tissue microenvironment.

3.2. Article II: Original article

PERICYTES INDUCED BY COLD EXPOSURE IN DIFFERENT ADIPOSE TISSUES OF MICE

Caroline C. Picoli, Michele Macedo Moraes, Abdulhakeem biola ajibike, Bryan Ôrtero Perez Gonçalves, Gabryella S. P. Santos, Beatriz G. S. Rocha, Alinne C. Costa, Leda Maria de Castro Coimbra Campos, e Alexander Birbrair

Abstract

Cold exposure induces browning of white adipose tissue (Saito et al.), increase in brown adipose tissue, with consequent tissue remodeling due to changes in vascular density. Pericytes are multipotent vascular progenitors that may be essential for growth and preservation of blood vessel integrity. For analysis, we used NG2-DsRed/Nestin-GFP mice, which could allow the identification of pericyte subtypes. We discovered the presence of two subsets of pericytes in various WAT and BAT deposition, the NG2+/Nestin- (type-1) and the NG2+/Nestin+ (type-2). After cold exposure, we observed a significant increase in type-1 cells in all adipose tissues and increase in type-2 in adipose tissues more susceptible to phenotypic changes from cold exposure. We also found that among the markers, several cells expressed only the marker Nestin+, and that some of them appeared associated with blood vessels, but a small amount were not associated with blood vessels. Here, different subsets of perivascular cells were revealed in the adipose tissue (WAT and BAT), which increased significantly after two weeks of cold exposure (8ºC).

Introduction

Adipose tissue is an essential component of metabolic control (Spiegelman & Flier, 2001), playing a key role in maintaining energy homeostasis in several species, due to its main function which is to store lipids in a unilocular manner in adipocytes called white (Henry et al., 2012; Sanchez-Gurmaches & Guertin, 2014). However, with the description of the uncoupling protein 1 (UCP1) present in the inner membrane of mitochondria, described around 1985 (de Jong, Larsson, Cannon, & Nedergaard, 2015), it was observed that these white adipocytes could undergo a process of transdifferentiation, called browning, that is, the white adipocytes taking on phenotypic characteristics of brown adipose cells, which are specialized in dissipating heat, later being called beige adipose cells (Bostrom et al., 2012).

In the microenvironment of adipose tissue, the vascularization plays an important role in maintaining the plasticity of adipose mesenchymal and regulating tissue angiogenesis (Panina et al., 2018; Rupnick et al., 2002). The angiogenic process seems to provide conditions for adipogenic progenitor cells to differentiate as well (Farrington-Rock et al., 2004; W. Tang et al., 2008). Furthermore, several studies have suggested that adipogenic progenitors are perivascular stem cells (Cai et al., 2011; Lin et al., 2008; Rodeheffer et al., 2008; W. Tang et al., 2008). Thus, pericytes, which are perivascular cells, could potentially be involved in the origin of mature adipocytes (Tang et al., 2008; Tran et al., 2012).

Birbrair and colleagues demonstrated the presence of skeletal muscle pericyte subtypes 1 and 2, with distinct roles, related to adipogenic and fibroblastic potential; and to myogenic, vascular, and neuronal potential, respectively (Birbrair et al., 2017), and this generated a discussion about the plasticity of pericytes in several other tissues (Birbrair et al., 2017; Guimaraes-Camboa et al., 2017). Therefore, verifying and quantifying the contribution and potential of pericytes in various organs and tissues, under physiological and pathological conditions, is still necessary.

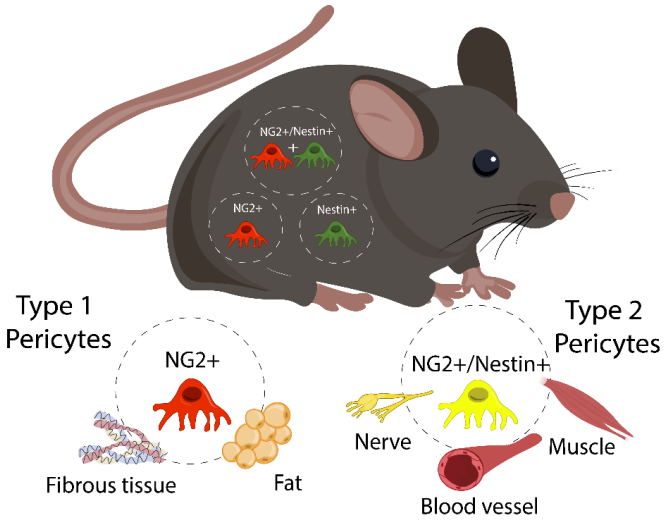
Cold exposure is known as a potent stimulator of tissue remodeling, which can be essential for health maintenance, and can be considered as a therapeutic bias for several metabolic diseases that include obesity, type 2 diabetes (Bartelt & Heeren, 2014; J. Wu, Cohen, & Spiegelman, 2013) and cancer (Seki et al., 2022). Cold causes potent changes that lead to remodeling of the vascular structure (Cypess et al., 2012; Lim et al., 2012; Prior, Yang, & Terjung, 2004), increasing angiogenesis, with consequent

increased vascularization of organs and tissues, elevating oxygenation and nutrient supply, due to increased blood flow (Bloor, 2005; Yamanishi et al., 2006).

In this study, we tested the hypothesis that 2-week cold exposure, at 8ºC, contrasting the room temperature group (26ºC), could promote increased the population of pericytes in different adipose tissues. For this, we used the NG2-DsRed+/Nestin-GFP+ model that allows localization and quantification of type-1 pericytes (NG2+/Nestin-) and type-2 pericytes (NG2+/Nestin+). We found that 2-weeks cold exposure increased type-1 pericytes in all adipose deposits, while type-2 pericytes increased only in the iWAT and BAT deposits. These data sets will be useful for further investigation into how type-1 and type-2 pericytes influence adipose tissue plasticity.

Materials and methods

Ethical issues and animal procedures. The Animal Care and Use Committee at Universidade Federal de Minas Gerais approved animal handling and procedures in this work 15/2022, and all experiments were performed according to the ARRIVE guidelines. Our Nestin-GFP+ transgenic mice colony was maintained homozygous for the transgene on the C57BL/6 genetic background (Birbrair, Zhang, Wang, Messi, Enikolopov, et al., 2013; Birbrair, Zhang, Wang, Messi, Mintz, et al., 2013) (Figure Supplementary 1). Nestin-GFP+ mice were crossbred with NG2-DsRed+ mice to generate Nestin-GFP+/NG2-DsRed+ double-transgenic mice. Accordingly, this model presents endogen fluorescence for two proteins: Nestin-GFP+/undiferentiated cells (marked in green) and NG2- DsRed+/pericytes (marked in red). All animals were housed in controlled conditions for temperature (26 °C or 8 °C, according to experimental procedure) and under a 12:12-h light–dark cycle and fed ad libitum. Eight to ten-week-old male, Wild type (WT) and Nestin-GFP+/NG2-DsRed+ mice were used in this study. A fragment of the mice ear was taken for confirmation of the fluorescent phenotype by fluorescence microscope Invitrogen™ EVOS™ FL Imaging System (Model: AMF4300).



Supplementary Figure 1 – Representative scheme of the pericyte subtypes described by Birbrair and colleagues (Birbrair, Zhang, Wang, Messi, Mintz, et al., 2013). We see two subpopulations of pericytes associated with blood vessels, type-1 (NG2-DsRed+/Nestin-GFP-), associated with fibrosis accumulation and adipose tissue deposition and type-2 (NG2-DsRed+/Nestin-GFP+), associated with nerve, muscle, and blood vessel formation.

Design experimental

Cold Exposure Protocol. Male mice, age-matched 8- to 10- week-old, were kept in individual boxes and underwent a cold adaptation period for 7 days at 18°C. Immediately, after adaptation, the mice were taken to a continuous cold chamber at 8°C for 14 days 12 of all animal experiments is provided in figure 1 A. All mice were used and were randomized and divided into two groups: 1. Room temperature (RT) and 2. Cold exposure.

Temperature. The temperature inside the box of each mouse and the body temperatures of the thorax skin and tail skin were measured using an infrared thermometer (Fluke 566, Fluke Corporation, OH, USA, accuracy ± 1.0°C) positioned at a maximum vertical distance of 2 cm from the measurement point and with the aid of the laser sight of the thermometer (located 2.5 cm from the center of the infrared sensor, 2 cm in diameter). The thermal images were taken using a Professional Grade Thermographic Camera for Smartphones (FLIR ONE Pro). The analyses and images were performed in the program Flir Thermal Studio. To avoid circadian effects on body temperatures, measurements were conducted at the same time of day for all groups and times, between 11:00 a.m. and 12:00 a.m.

Parabiosis Surgery. For parabiosis, age-matched 8- to 10-week-old male mice wild-type (WT) (recipient) and Nestin-GFP/+NG2-DsRed+ (donor) were anesthetized with a mixture of 114 mg/kg ketamine and 17 mg/kg xylazine, and the surgery was performed as previously described (Kamran et al., 2013; Ruckh et al., 2012). Briefly, a longitudinal skin incision was performed from the elbow to the knee joint. Following the incision, we detached the skin from the subcutaneous fascia. Then, after exposing the underlying tissue, we sutured, using a double knot, the left elbow of one animal to the right elbow of the other. Similarly, we also performed the same suture between animals' knee joints to complete their attachment (Kamran et al., 2013). Finally, the skins of the two animals from the elbow to the knee were sutured using a 4–0 nylon suture.

Collection of tissue samples. At the end of the experimental protocols, the animals were weighed and anesthetized with a mixture of ketamine (80 mg/kg per body weight of each animal) and xylazine (15 mg/kg per body weight of each animal), administered intra-peritoneally, and then the animals underwent a median laparotomy to remove the white subcutaneous inguinal (iWAT), retroperitoneal (rpWAT), perigonadal (pWAT), and brown adipose tissue interscapular (BAT).

Immunohistochemistry and Microscopy. For immunofluorescence, the adiposes tissues from NG2-DsRed+/Nestin-GFP+ and all parabionts were fixed overnight at 4 °C in 4% buffered paraformaldehyde (PFA, pH = 7.4). This was followed by incubation with sucrose 30% for 48 hours, diluted in PBS, then embedded and frozen in the optimal cutting temperature compound (Khennouf et al.) (Tissue-Tek; Sakura-Finetek, Torrence, CA, USA). Embedded tissues were stored at -20 °C. 12 μm sections were sectioned in a CM1850UV cryostat (Leica Biosystems, Buffalo Grove, Illinois, USA), blocked for 90 min with 4% BSA in PBS + 0.5% Triton X-100 (Sigma, St. Louis, Missouri, USA) and immunolabelled with the following antibodies: Rat anti-CD31 (PECAM) (1:100, cat.# 14-0311-82; ThermoFisher, San Diego, CA, USA), an endothelial marker, Rabbit anti-Perilipin (1:100, cat. #9349; Cell Signaling); Rabbit anti-PDGFRβ; BioLegend, San Diego, CA, USA), Rabbit anti-T.H. cat. #PA14679 ThermoFisher, San Diego, CA, USA). Sections were counterstained with DAPI and sealed with Dako fluorescence mounting medium (Santa Clara, CA, USA). Stained sections were imaged at an inverted Zeiss LSM 880 confocal microscope (Oberkochen, Germany). For each animal we obtained at least

10 captures, at 20 x or 40 × magnification, from randomly selected fields from each section, avoiding any overlaps. The numbers of NG2-dsRed+, Nestin-GFP+, CD31+, PDGFRβ +, T.H.+ and DAPI+ cells were quantified using Fiji software® version 1.53.

Statistical analysis. The samples were calculated using the G*-power software (v.3.1.9.2) (Faul et al., 2007) that demonstrated the need for 9 samples per group to maintain the main immunofluorescence variable of NG2-DsRed+/Nestina-GFP+, the mean effect size was set at 0.25, P 0.05 and 80% power. The Shapiro-Wilk test was performed to verify the normality of the data. For parametric data unpaired Student's t-tests was used to identify differences between two independent groups. The α level was set at 0.05. Data are shown as mean ± standard error (SEM). All statistical analyzes were performed using the GraphPad Prism 8.0 software (GraphPad Software, San Diego, CA).

Results

Phenotypic changes of 2-weeks of cold exposure in adipose tissue

To explore the role of pericytes in adipose tissue, here we used double transgenic NG2-DsRed+/Nestin-GFP+ mices (Figure 1A) which have been through a one-week adaptation period at 18 ± 1.5 ºC and cold exposure for 2 weeks at 8 ± 1.6 ºC. Both experimental groups, showed no significant differences in body composition (g), nor for water intake and standard food intake (g) (Figure B, C and D). It is possible to notice significant differences in body temperature, tail and sawdust/box, throughout the experimental period, from adaptation to 18ºC to the cold exposure proper of 8ºC (Figure E, F and G).

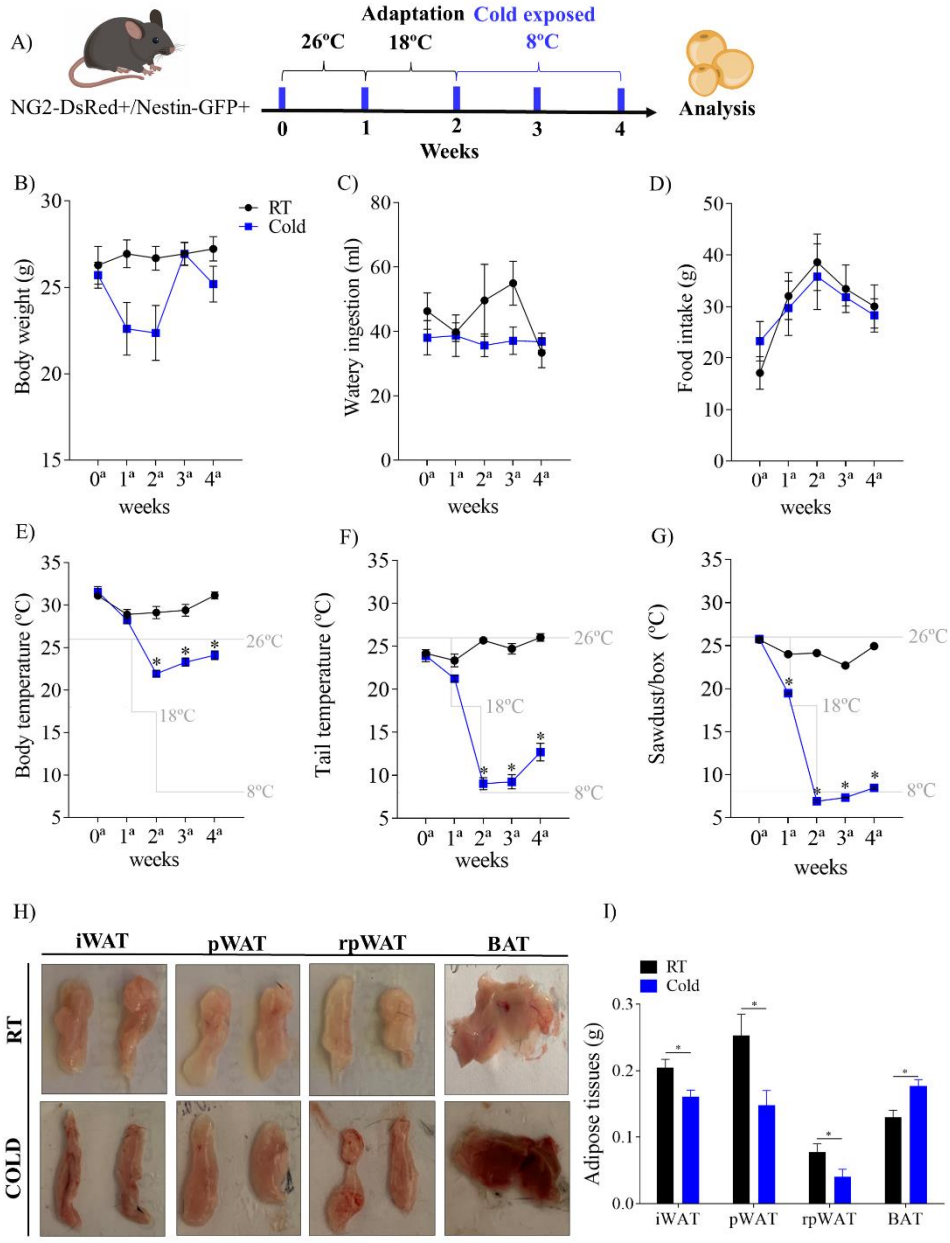


Figure 1 - Characterization of the 2-week cold exposure model in NG2-DsRed+/Nestin-GFP+ animals. A) Schematic diagram of the 2-week cold induction protocol. B) Body weight (g); C) Water intake, D) Food intake, E) Body temperature; F) Tail temperature, and G) Wood shavings, H) Representative pictures of adipose tissues: iWAT, pWAT, rpWAT and BAT, I) Weight of adipose deposits (g). Room Temperature (RT) (n=10); Cold (n=10). Statistical analysis: unpaired Student's t-tests. * $p < 0.05$ Significant difference between groups. Data are mean ± SEM.

After the 2-week cold exposure (8°C) we also observed a visible phenotypic change in iWAT, pWAT, rpWAT and BAT adipose tissues (Figure H), with a consequent significant reduction in the weight of all WAT analyzed and a significant increase in BAT (Figure I).

There is strong evidence that cold exposure can increase the appearance of small lipid droplets that change the appearance of white adipocytes from unilocular to multilocular, which increases their ability to supply substrate to other tissues (Sepa-Kishi, Jani, Da Eira, & Ceddia, 2019). Thus, we initially evaluated the area of the adipocytes of both groups and observed that there was a significant reduction in adipocyte area (Figure 2 A and B), for all the analyzed deposits, after 2-week cold exposure. Moreover, we checked the expression of perilinin, a marker of lipid droplets, in the NG2-DsRed+/Nestina-GFP+ mice. We found that after cold exposure, there was a significant increase in perilipin expression (Figure 2 C and D) in all adipose tissues of the mice that were exposed to cold compared to the RT group.

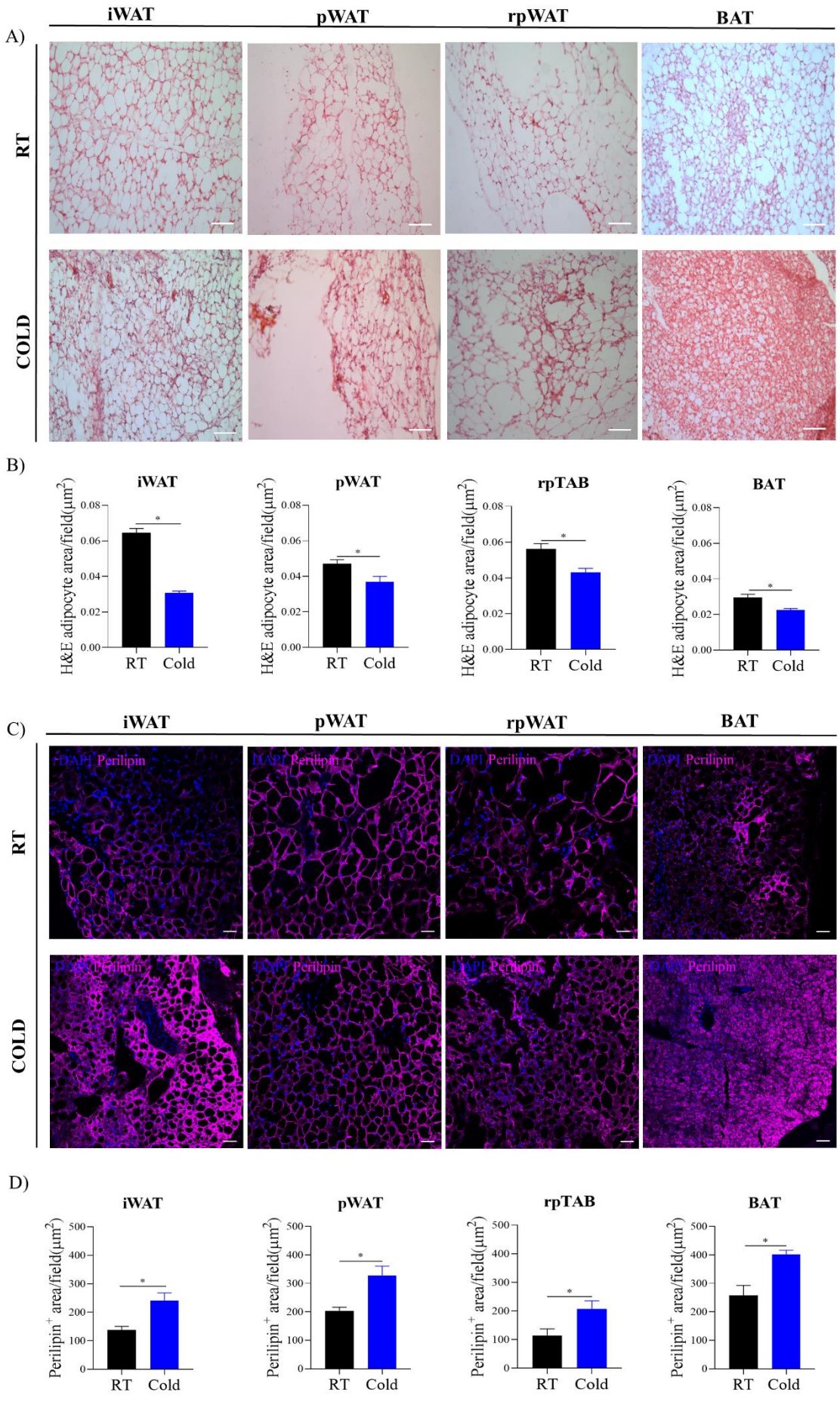

A)
iWAT
pWAT
rpWAT
BAT
RT
COLD
B)
iWAT
pWAT
rpTAB
BAT
H&E adipocyte area/field(μm²)
0.08
0.06
0.04
0.02
0.00
RT
Cold
*
C)
iWAT
pWAT
rpWAT
BAT
RT
COLD
DAPI Perilipin
D)
iWAT
pWAT
rpTAB
BAT
Perilipin⁺ area/field(μm²)
500
400
300
200
100
0
RT
Cold
*

Figure 2 - Decreased adipocyte area and increased perilipin in WAT and BAT after 2-week cold exposure. A) H&E of adipose tissues, B) Adipocytes areas quantification in iWAT, pWAT, rpWAT and BAT after 2-week cold exposure; C) Perilipin was detected by immunofluorescence, D) Perilipin quantification in iWAT, pWAT, rpWAT and BAT, adipose tissues after 2-week cold exposure. Room Temperature (RT) (n=5); Cold (n=5). Statistical analysis: unpaired Student's t-tests. * $p < 0.05$ Significant difference between groups. Data are mean ± SEM. Scale Bar, 50 μm.

Cold-induced increase in vascular density and pericyte subpopulation markers

It has been shown previously in other studies that cold stimulation is able to increase tissue angiogenesis (Luo et al., 2017; Xue et al., 2009), and can also increase adipogenic progenitors (D. C. Berry et al., 2016; Y. H. Lee et al., 2015). To determine the potential effects of cold exposure on adipose tissue vasculature, we compared mice that were kept at RT (26ºC) or cold exposure for 14 days. We made a histological analysis, using an immunofluorescence, and verified a higher labeling of positive cells expressing CD31 in iWAT, pWAT and BAT, while for rpTAB tissue we observed no significant difference (Figure 3 A and B).

Some studies have shown that there is a heterogeneity of pericytes in several tissues , as in the spinal cord (Goritz et al., 2011) skeletal muscle (Birbrair, Zhang, Wang, Messi, Enikolopov, et al., 2013), retina (Trost, 2019 #459), and others organs. In adipose tissue, it has been shown that these cells may be related to the development of mature adipocytes (W. Tang et al., 2008). To investigate whether 2 weeks of cold exposure mobilize the subtypes of pericytes cell in adipose tissue, we verify type-1 (NG2-DsRed+/Nestin-GFP-) and type-2 (NG2-DsRed+/Nestin-GFP+) pericyte subpopulations in Fig 3. and evaluated histological sections of different adipose tissue depots (iWAT, pWAT, rpWAT and BAT). Our analysis at 2 weeks after cold exposure reveals that type-1 pericytes increased significantly in all quantified adipose tissues, whereas only type-2 pericytes increased significantly only in iWAT and BAT adipose tissues (Figure 3C). We also analyze the increase in percentage of type-1 (113,8% in iWAT, 119,5% in pWAT, 131,9% in rpWAT and 217,8% in BAT) and type-2 (86,1% in iWAT, 51,4% in pWAT, 44,2% in rpWAT and 263,7% in BAT).

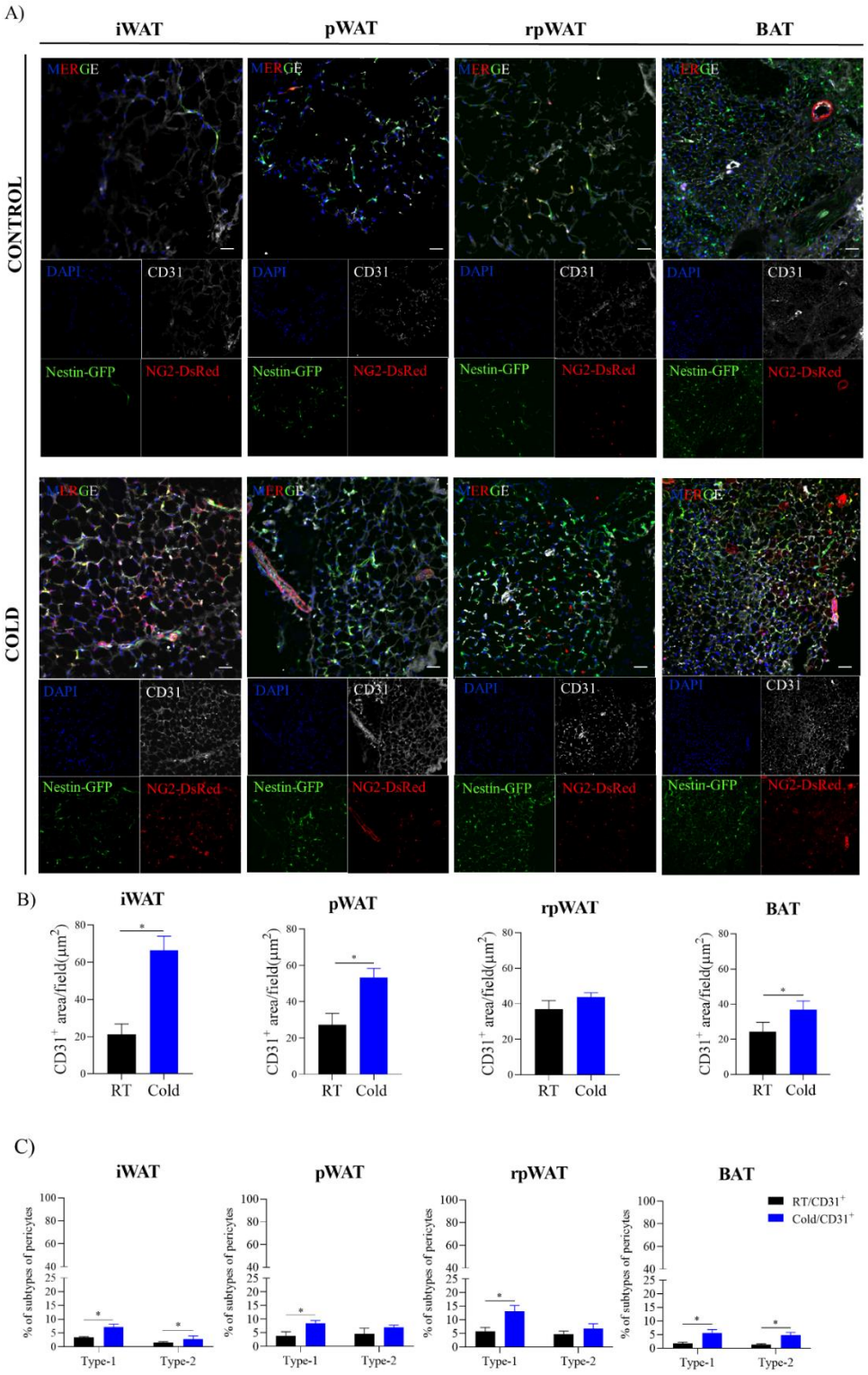


Figure 3 - Increased vascular density and type-1 and type-2 pericytes after 2-week cold exposure. A) Representative immunofluorescence images of NG2-DsRed+/Nestin-GFP+ mouse adipose tissue sections labeled for endothelial cells (CD31;gray), type-1 pericytes (NG2-DsRed+/Nestin-GFP-; red) and type-2 pericytes (NG2-DsRed+/Nestin-GFP+; yellow) and nuclei (DAPI; blue). Quantification of B) CD31+ per area/field and C) The percentage of type-1 and type-2 pericytes after 2-week cold exposure, from iWAT, pWAT, rpWAT and BAT adipose

tissues. Room Temperature (RT) (n=5); Cold (n=5). Statistical analysis: unpaired Student's t-tests. * p <0.05 Significant difference between groups. Data are mean ± SEM. Scale Bar, 50 µm.

We verified the expression of PDGFRβ+, a marker that is almost restrictively expressed in perivascular mesenchymal cells, encompassing smooth muscle cells and pericytes (Andrae, Gallini, & Betsholtz, 2008; Heldin & Westermark, 1999; C. Sun et al., 2020; Vishvanath et al., 2016). We have found that for all adipose tissue (iWAT, pWAT, rpWAT and BAT) there was a significant increase in the PDGFRβ+ marker after 2 weeks of cold exposure compared to the RT group (Figure 4 B). We could also observe (under the merge) that the PDGFRβ+ marker had a high co-labeling with the other type-1 (RT = 98,9 ± 0.2 % and Cold = 99.9 ± 0.1 %; NG2+ cells were positive for PDGFRβ+) and type-2 pericyte markers (RT = 99.2 ± 0.1 % and Cold = 99.8 ± 0.2 % ; NG2+/Nestin+ cells were positive for PDGFRβ+) (Figure 4 A).

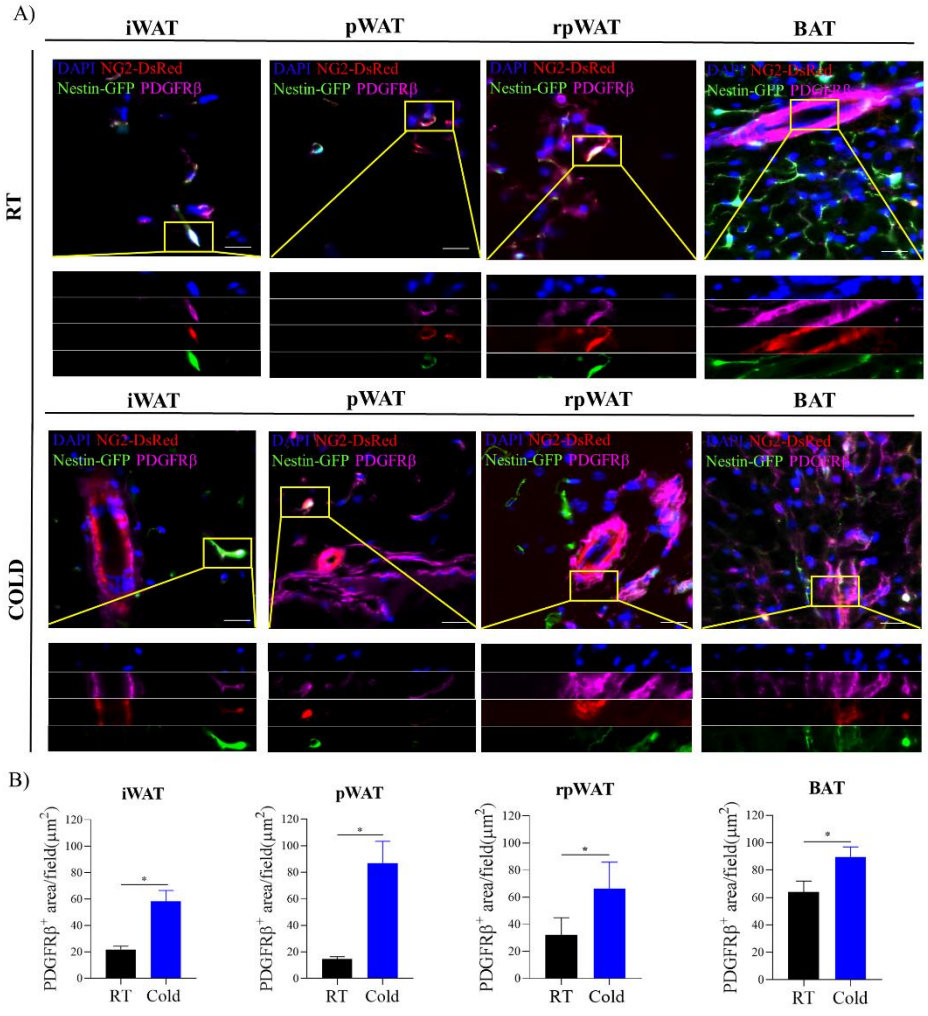


Figure 4 - Presence of PDGFRβ+ in adipose tissues of NG2-DsRed+/Nestina-GFP+ mice after 2-week cold exposure. A) Representative immunofluorescence images of adipose tissue sections from NG2-DsRed+/Nestina-GFP+ mice labeled for PDGFRβ (magenta) and nucleus (DAPI; blue). Quantification of PDGFRβ+ per field (μm2), from adipose tissues B) iTAB, pTAB, rpTAB and TAM. Room Temperature (RT) (n=5); Cold (n=5). Statistical analysis: unpaired Student's t-tests. * $p < 0.05$ Significant difference between groups. Data are mean ± SEM. Scale Bar, 50 μm.

Increased sympathetic innervation after 2-weeks of cold exposure

After 2-weeks exposure to cold, adipose tissue may undergo a " browning" process, which can be observed mainly in subcutaneous adipose tissue, leading to the appearance of multilocular adipocytes that are UCP-1 positive (Kajimura et al., 2015). WAT remodeling, which is often strongly related to microcapillary development and enhanced sympathetic innervation, has been connected to the emergence of beige adipocytes. (Cinti, 2001a). To determine if the 14-day cold exposure at 8°C was effective in our experimental technique, we used H.T. staining. By using the immunofluorescence approach, we saw that following exposure to cold, the T.H. in the iTAB, pTAB, and TAM tissues significantly increased as compared to the control group (Figure 5 A, B).

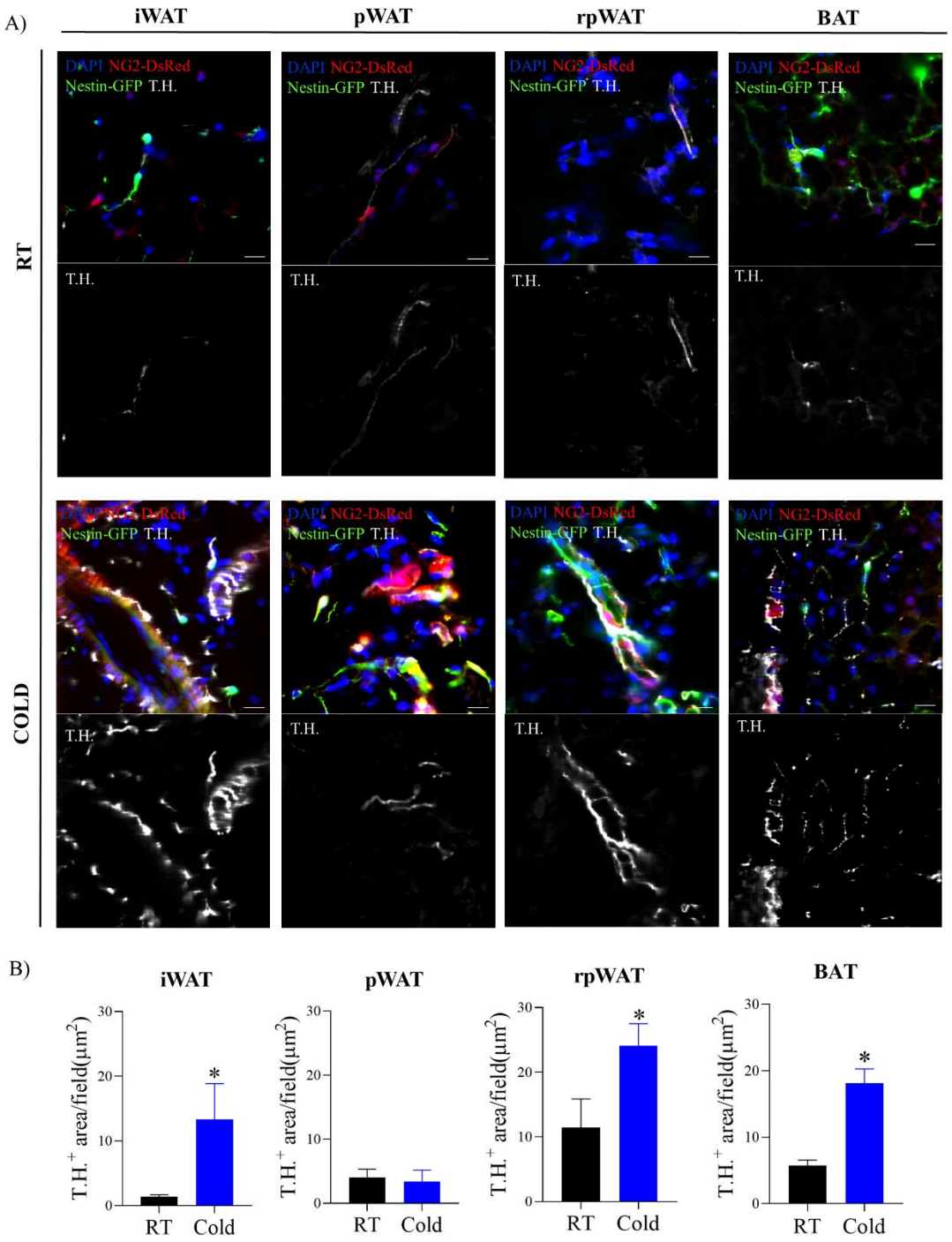


Figure 5 - Increase in sympathetic innervation, of NG2-DsRed+/Nestina-GFP+ mice after 2-week cold exposure. A) Representative immunofluorescence images of adipose tissue sections from NG2-DsRed+/Nestina-GFP+ mice labeled for sympathetic innervation (T.H; gray) and nucleus (DAPI; blue). Quantification of T.H.+ per field (µm2), from adipose tissues B) iWAT, pWAT, rpWAT and BAT. Room Temperature (RT) (n=5); Cold (n=5). Statistical analysis: unpaired Student's t-tests. * p <0.05 Significant difference between groups. Data are mean ± SEM. Scale Bar, 50 µm.

2-weeks of cold exposure increase the number of Nestin-GFP+ cells associated and not-associated with blood vessel in adipose tissue

We observed that in addition to type-1 and type-1 pericytes, there was a revealed number of Nestin-GFP cells in the adipose tissues (Fig 6 A). To examine the localization of Nestin-GFP+ cells in adipose tissue in relation to the vasculature, we immunized sections of the different adipose depots of NG2-DsRed+/Nestin-GFP mice with CD31 antibody, a marker for endothelial cells. We revealed that the percentage of Nestin-GFP+ cells associated with blood vessels increased substantially in all depots of mice subjected to cold, as did the percentage of Nestin-GFP+ cells not associated with blood vessels, although in a smaller proportion (Fig 6 B and C).

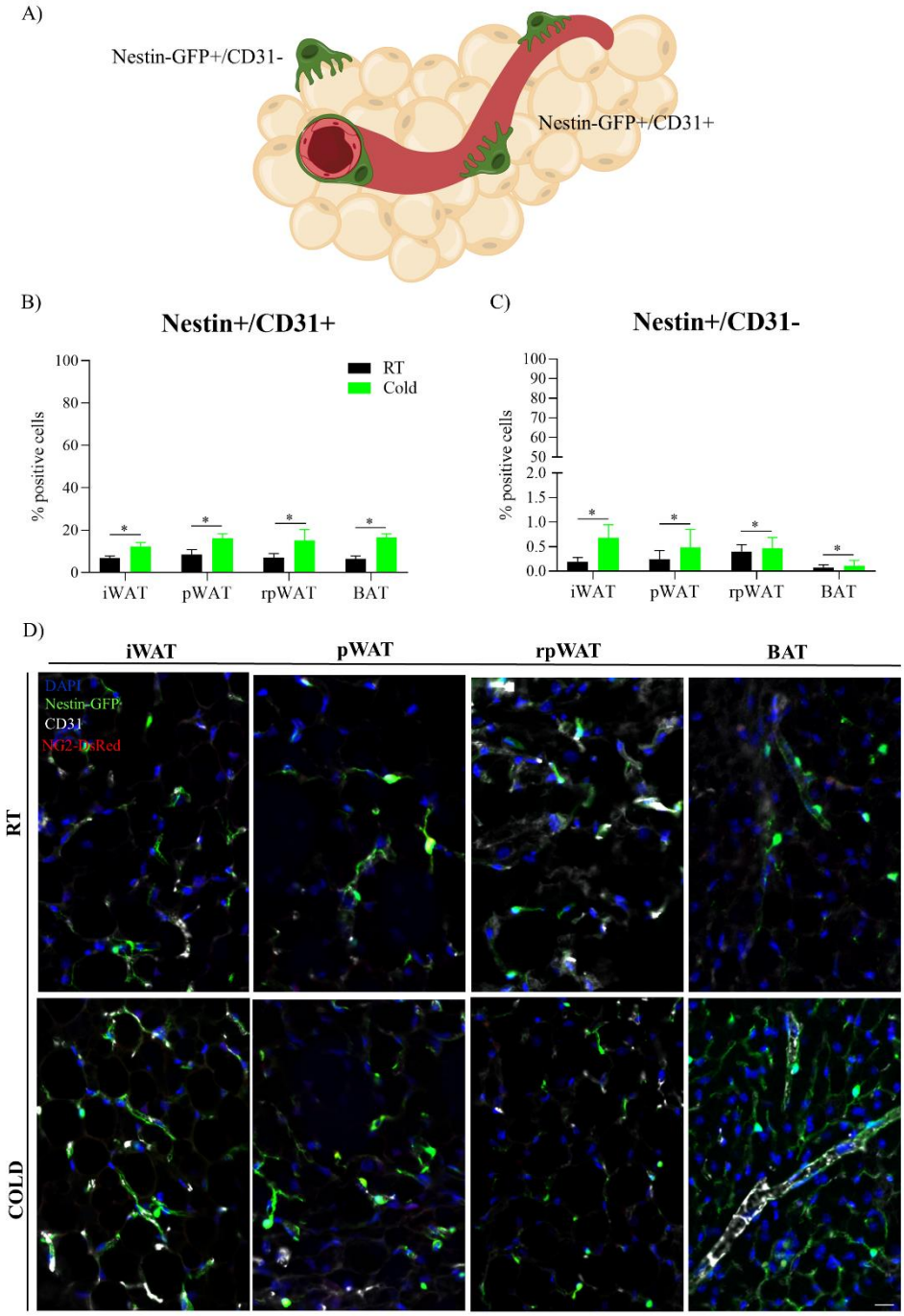
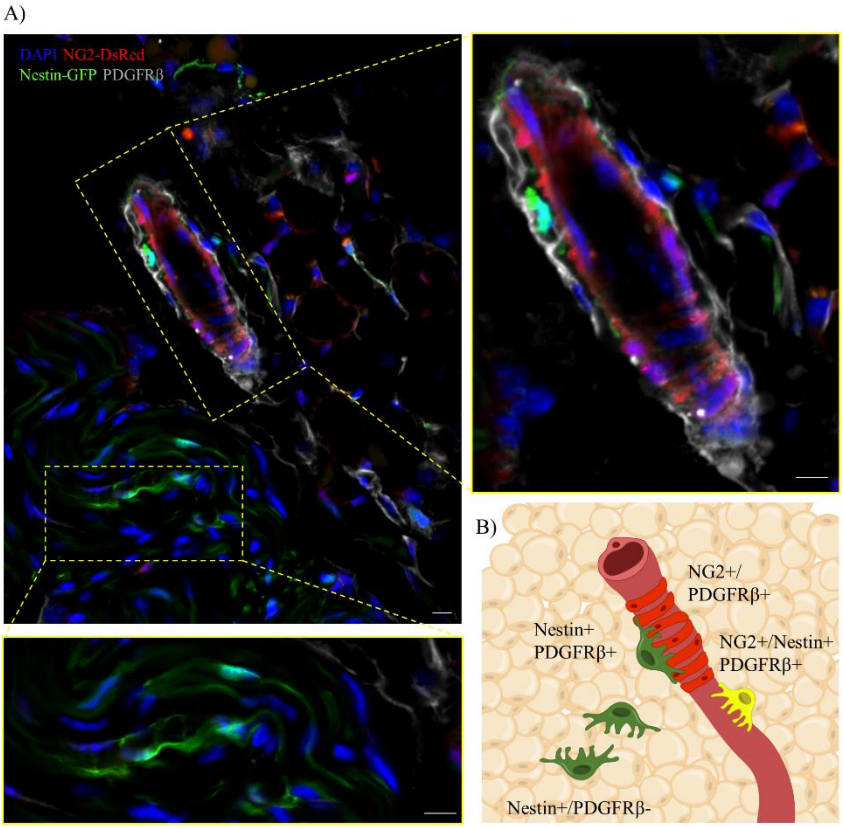


Figure 6 - Nestin-GFP+ cells increase in the adipose tissue after 2-weeks of cold exposure. A) Representative scheme of Nestin-GFP cells associated to blood vessels (CD31+) or not associated (CD31-); B) Quantification of Nestin-GFP+/CD31+ and Nestin-GFP+/CD31- per field (µm2), C) Representative immunofluorescence images of adipose tissue sections from NG2-DsRed+/Nestina-GFP+ mice labeled for blood vessels (CD31; gray) and nucleus (DAPI; blue) from adipose tissues iWAT, pWAT, rpWAT and BAT. Room Temperature (RT) (n=5); Cold (n=5). Statistical analysis: unpaired Student's t-tests. white arrows = indicate Nestin-GFP cells not associated with blood vessels; * p <0.05 Significant difference between groups. Data are mean ± SEM. Scale Bar, 50 µm.

In addition, this small percentage of Nestin+ cells non-associated with blood vessels, after 2-weeks cold exposure, also did not co-labeling with another pericyte marker PDGFRβ+ (Supp. Fig. 2 A and B). Thus, the cold exposure increased considerably, which suggests that after the cold the Nestin-GFP+ cells are also recruited to the adipose tissues (Fig 6 D).



Supplemental Figure 2. Schematic illustration of BAT Nestin-GFP+ cells not co-labeling with PDGFRβ+ after 2-weeks cold exposure. Schwann cells detach and migrate away from the nerve fibers, associating to blood vessels within the adipose tissue microenvironment. Scale Bar, 10 µm.

Circulating Cells are Responsible for the Increase in Nestin-GFP+ Cells after 2-weeks of cold exposure

To reveal the origin of the Nestin-GFP+ cells that had been increased in adipose tissues after cold, we made a parabiosis model (Fig 7 A) to examine whether the contribution of these cells could come from the circulation, since it has already been shown that Nestin-GFP cells can be recruited in other experimental models, performed by our group (Coimbra-Campos, 2021 #334). WT and NG2-DsRed/Nestin-GFP mice were conjoined as described in the Methods section. Shared circulation was checked 30 days after parabiosis surgery to proceed with the cold exposure protocol (Fig 7 B). Mice

were euthanized after this protocol. For the iWAT we had opterd to make a division of the analysis into proximal and distal inguinal (iWATp and iWATd), since during the conjoined of the parabiosis pairs, we observed that the medial skin junction of each animal would end up having a possible contact.

Both experimental groups, showed no significant differences in body composition (g), nor for water intake and standard food intake (g) (Figure7 C, D and E). It is possible to notice significant differences in body temperature, tail and sawdust/box, throughout the experimental period, from adaptation to 18ºC to the cold exposure proper of 8ºC (Figure F, G, and H). We also used an Infrared Thermal Image of the parabiosis pairs over weeks of cold exposure (Fig 7 I), to demonstrate the cold acclimation process, that which coincides with the results observed in the measurements. In addition, the weight of the adipose tissues was quantified (in pairs) after 2-weeks cold exposure and we observed significant reduction in all WAT, with a significant increase in BAT (Fig 7 J), similar to the results we observed in Fig. 1 I.

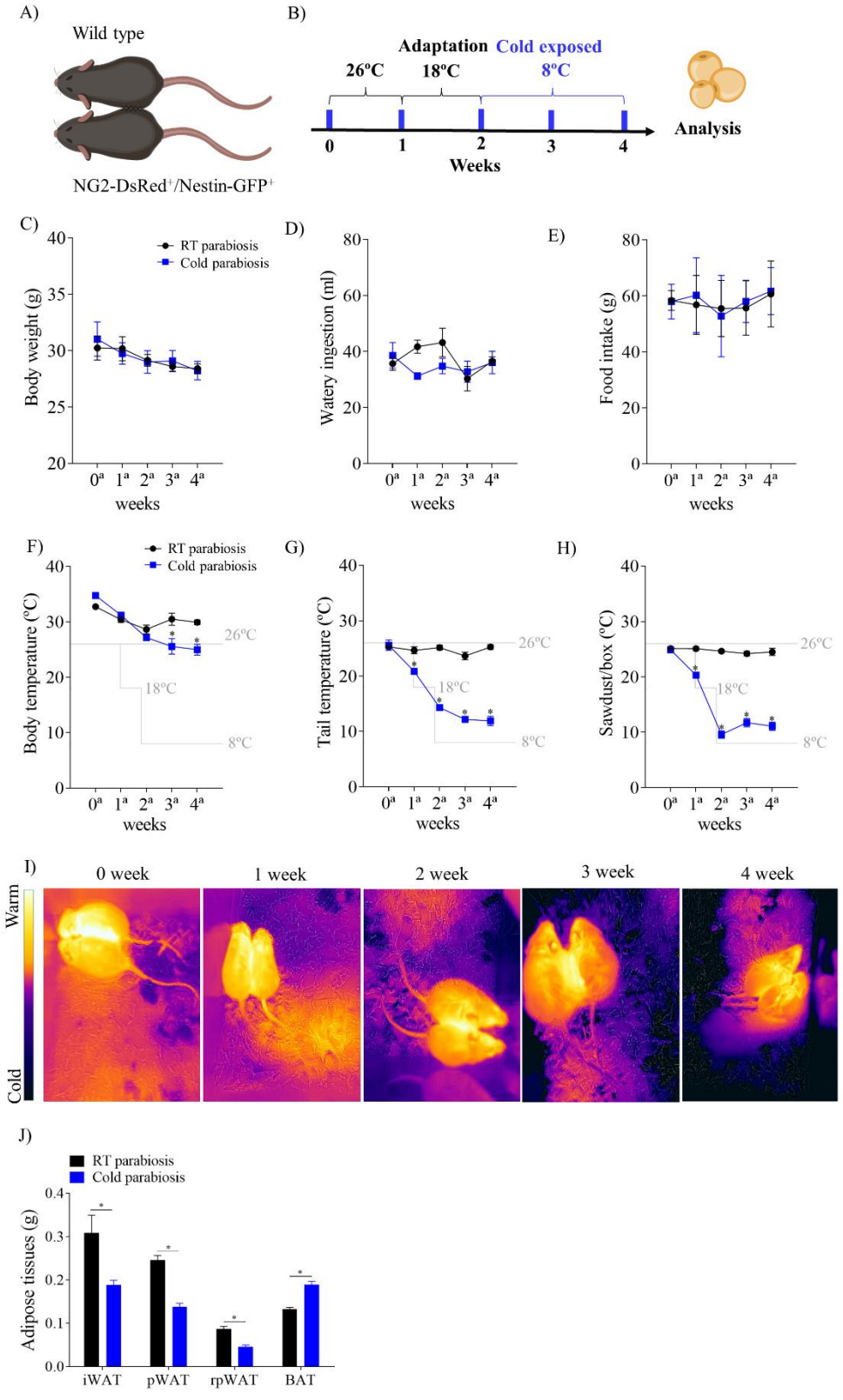


Figure 7 - Characterization of the 2-week cold exposure model in parabiosis WT with NG2-DsRed+/Nestina-GFP+ mices. A and B) Schematic diagram of the 2-week cold induction protocol. C) Body weight (g); D) Water intake, E) Food intake, F) Body temperature; G) Tail temperature, and H) Sawdust/box, I) Representative schematic of Infrared Thermal Image of the

parabiosis pairs; and J) Weight of adipose deposits (g). Room Temperature (RT) (n=4); Cold (n=4). Statistical analysis: unpaired Student's t-tests. * p <0.05 Significant difference between groups. Data are mean ± SEM.

Endogenous fluorescence analysis of adipose tissues from parabiosis pairs of WT mice with NG2-DsRed+/Nestin-GFP+ demonstrated that for iWATp there was a possible migration of NG2-DsRed+ and Nestin-GFP+ cells, which were more intensely marked after the 2-week cold exposure (Fig. 8 A). In other deposits analyzed, such as iWATd, pWAT and BAT, we observed weak Nestin-GFP labeling, with morphology similar to nerve fiber bundles (NFBs) (Stavely et al., 2022).

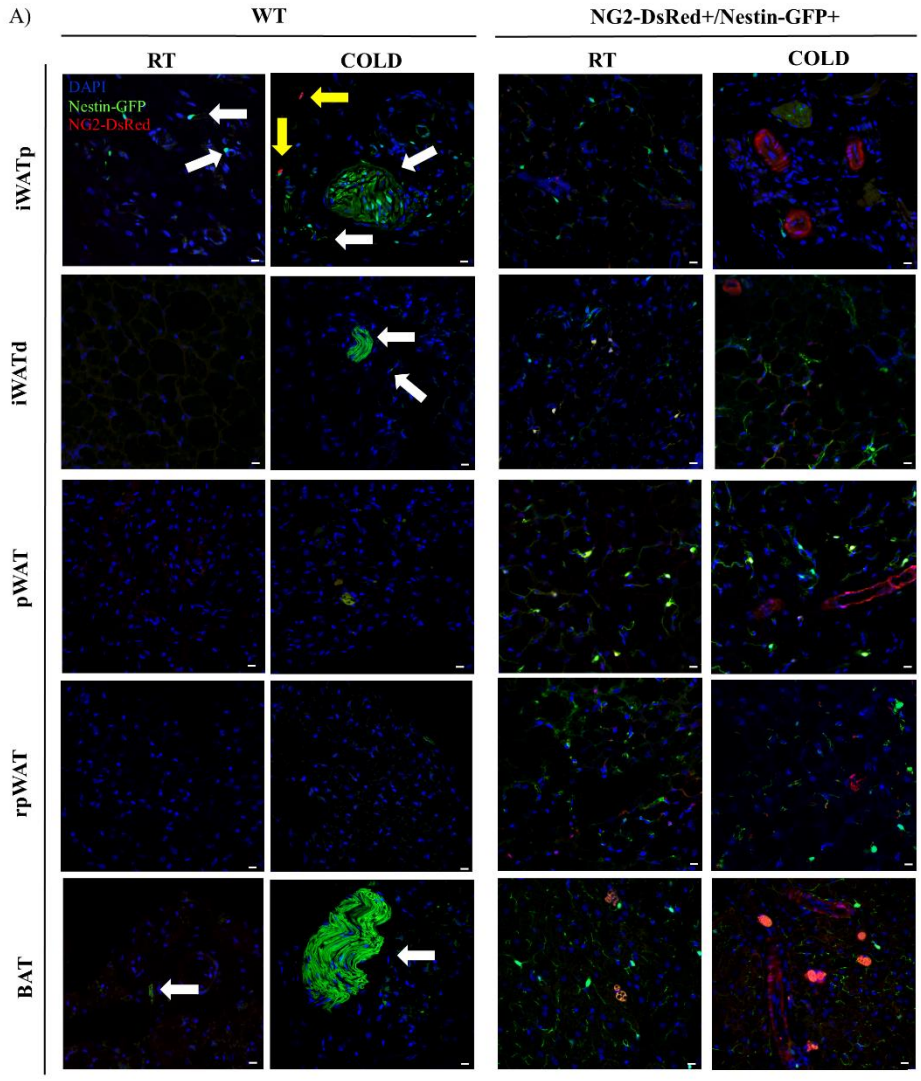


Figure 8 - Endogenous fluorescence analysis in parabiosis WT with NG2-DsRed+/Nestina-GFP+ mices after 2-weeks of cold exposure. A) Representative fluorescence of WT (Ivanova et al.) and NG2-DsRed+/Nestin-GFP+ mices in RT or Cold conditional. Nucleus (DAPI; blue) from adipose tissues iWAT, pWAT, rpWAT and BAT. Room Temperature (RT) (n=4); Cold (n=4). White arrows indicate Nestin+ cells; yellow arrows indicate NG2+ cells. Scale Bar, 10 µm.

To verify the percentage of cells of parabiosis pairs after cold exposure, we quantified the percentage of Nestin-GFP+ cells (Table 1) and observed that in WT animals there was an increase of these cells in iWAT tissues, mainly in iWATp due to contact, being less observed in iWAtd and BAT mice.

Table 1- Percentage of Nestin-GFP+ cell in adiposes tissue of parabiosis pairs after 2-weeks of cold exposure

		Parabiosis pairs (n=4)			
Mices		**WT**		**NG2-DsRed+/Nestin-GFP+**	
Experimental condition		*RT*	*Cold*	*RT*	*Cold*
% of Nestin-GFP cells	**iWATp**	0.8 ± 0.0	4.59 ± 1.2 *	4.42 ± 1.5	10.51 ± 0.7*
	iWATd	0.00 ± 0.0	1.32 ± 0.2 *	5.42 ± 1.5	12.03 ± 0.5*
	pWAT	0.00 ± 0.0	0.08 ± 0.1	4.25± 0.3	12.22 ± 1.5*
	rpWAT	0.00 ± 0.0	0.02 ± 0.00	3.12 ± 0.6	8.32 ± 0.9*
	BAT	0.09 ± 0.0	2.34 ± 1.1 *	5.98 ± 1.3	9.24 ± 0.6*

Temperature (RT) (n=4); Cold (n=4). Statistical analysis: unpaired Student's t-tests. * $p < 0.05$ Significant difference between groups. Data are mean ± SEM.

Discussion

To verify whether 2-weeks cold exposure affect different populations of pericytes could be present in WAT and BAT deposits, we explore some immunohistochemistry analysis of double transgenic NG2-DsRed+/Nestin-GFP+ mice.

Focusing on the cellular subpopulation of pericytes previously characterized by Birbrair et al. we use the definition of pericytes type-1 (NG2-DsRed+/Nestin-GFP-) and type-2 (NG2-DsRed+/Nestin-GFP+) (Birbrair, 2019; Birbrair et al., 2017; Birbrair, Zhang, Wang, Messi, Enikolopov, et al., 2013; Birbrair, Zhang, Wang, Messi, Mintz, et al., 2013; Birbrair et al., 2015). Our finding suggests that type-1 and type-2 pericytes can be found in all adipose tissue of mice, representing a small fraction of this cell type, in adipose microenvironment, under normal conditions, that after cold exposure increased in tissues more susceptible to stimulation (iWAT and BAT) (Fig 3 A and C).

Cold exposure of mice is a conventional method to stimulate BAT activity and induce browning of WAT that has beneficial effects on whole-body lipid metabolism (Bartelt & Heeren, 2014; Bostrom et al., 2012; Cypess et al., 2012; Cypess et al., 2015; De Matteis et al., 2013; Vitali et al., 2012). In addition, changes in the composition of adipose tissue deposits can be observed, with a reduction in WAT and a consequent increase in BAT, because cold exposure alters the overall composition of lipid classes, leading to changes (Xu et al., 2019).

The adipose tissues in order to maintain body temperature, especially when cold exposure occurs, tend to increase the expression of UCP1, in the mitochondria, with high levels of β-oxidation of free fatty acids, which will be hydrolyzed from triglycerides located into lipid droplets (Benador et al., 2018; Xu et al., 2019; Yu et al., 2015), Thus, increased perilipin expression in WAT and BAT during the experimental protocol is critical for heat production. This is because, it has been observed that perilipins, which are support proteins for lipid droplets, are potentially candidates to play a role in interacting with mitochondria for shiver-free heat production (H. Wang et al., 2011).

For adipose tissue remodeling to occur, angiogenesis is a process that must be required, besides being an important reservoir of adipogenic progenitors (Lim et al., 2012; Luo et al., 2017; W. Tang et al., 2008; Tran et al., 2012). We observed in our study that 2-week cold exposure increases vascular density all adipose tissues, this is occur, because some authors have already observed that acute or chronic cold exposure

is able to induce pro-angiogenic factors (Asano, Morimatsu, Nikami, Yoshida, & Saito, 1997; Fredriksson, Nikami, & Nedergaard, 2005; Lim et al., 2012; Luo et al., 2017).

It has been shown previously that pericytes have an adipogenic potential and therefore may potentially be involved in the origin of mature adipocytes (W. Tang et al., 2008; Tran et al., 2012). In addition, there seems to be heterogeneity among the pericytes present in the same tissue (Birbrair et al., 2017), and it is still necessary to better evaluate the potential and heterogeneity of these cells in different adipose deposits, as well as in other tissues and conditions (Guimaraes-Camboa et al., 2017).

We observed that NG2+ cells (type-1 pericytes) increased in all adipose tissues with cold stimulation, whereas NG2+/Nestin+ cells (type-2 pericytes) increased only in iWAT and BAT tissues, which are more susceptible to stimulation. It is suggestive that these perivascular cells may behave as sentinels in some situations, either to increase tissue angiogenesis, or adipogenic progenitors. Previous observations have shown that NG2+ cells can constitute a population of mesenchymal progenitors capable of giving rise to mature adipocytes (D. C. Berry et al., 2016).

Evaluating whether all pericytes observed in adipose tissue, regardless of deposition, have equal differentiation potential to mature adipocytes, or beige adipocytes is a matter of debate, as well as for other tissues (Yianni & Sharpe, 2019). However, so far, this is the first study that differentiates between two subpopulations of pericytes in different deposits of adipose tissue type-1 (NG2+/Nestin-) and type-2 (NG2+/Nestin+).

Due to the heterogeneity of the pericytes, we used another marker of mural cells. The PDGFRβ, as this is one of the critical markers for the maintenance and localization of pericytes in endothelial cells (Lindahl et al., 1997). Vishvanath and colleagues (Vishvanath et al., 2016) evaluated PDGFRβ for mural cells, in which they performed lineage screening and demonstrated that these cells could contribute to the browning phenotype of adipose tissue due to multiple waves of beige adipogenesis either after cold induction, or white adipocyte adipogenesis after a high fat diet. Other lineage tracing studies in order to investigate adipogenic progenitors, used PDGFRα and PDGFRβ and observed that both markers contribute diversely to adipocyte differentiation, while PDGFRα was shown to be more involved with postnatal growth and adulthood, adipocytes derived from PDGFRβ positive mosaic showed a contribution only with postnatal growth. Furthermore PDGFRα deletion in adult animals increased adipogenesis and formation of beige adipocytes, which expressed

UCP1, after β3-adrenergic induction, whereas PDGFRβ deletion allowed increased adipogenesis of white, brown and beige adipocytes, thus demonstrating that a negative PDGFR relationship could be an important event in the transition from adipogenic progenitors to mature adipocytes (C. Sun et al., 2020).

Cinti has seen that the appearance of beige adipocytes has been associated with WAT remodeling, which is normally closely linked to microcapillary formation and increased sympathetic innervation (Cinti, 2001a). Some studies have shown that about 2% to 15% of adipocytes are observed in contact with sympathetic fibers in WAT (Vitali et al., 2012; Zeng et al., 2015). Furthermore, Jiang and colleagues using a 3D processing method, based on the iDISCO technique, (Belle et al., 2014; Renier et al., 2014) observed that sympathetic arborization in adipose tissue may be closely related to about 90% of the individual adipocytes present in the tissue (H. Jiang, Ding, Cao, Wang, & Zeng, 2017), being even greater than what is actually observed. Furthermore, when the authors used a pharmacological approach with 6-hydroxydopamine (6-OHDA), used to locally remove sympathetic fibers in subcutaneous adipose tissue, they observed that this sympathetic innervation is essential to maintain multilocular beige adipose cells after the 96-hour cold challenge (H. Jiang et al., 2017).

Sympathetic innervation can also have a close relationship with blood vessels (Chi et al., 2018). We observed that the majority of pericytes tipo-1 (NG2+/Nestin-) and pericytes tipo-2 (NG2+/Nestin+) cells in the different adipose deposits are associated with blood vessels, but it is still necessary to better evaluate the relation of these cells with the sympathetic innervation. Future studies should evaluate whether the release of neurotransmitters could drive the pericytes to differentiate into mature adipocytes (white, brown or beige), contributing to the phenotypic change due to cold stimulation.

After performing subtypes of pericytes comparisons, we observed that there was a massive increase in Nestin+ cells (Fig. 6) when compared to NG2+ cells only (Fig. 3). It has been shown before, that Nestin+ is also a marker for pericyte (Mignone, Kukekov, Chiang, Steindler, & Enikolopov, 2004) but has also shown potential as progenitor stem cells (Dore-Duffy et al., 2006; Mendez-Ferrer et al., 2010), and although Nestin+ expression has not been fully elucidated in WAT, Iwayama and colleagues evaluated by lineage screening that Nestin-Cre/TdTomato expression drives the promoter to the onset of adipogenesis in young mice that received a high-fat diet for 12 weeks (Iwayama et al., 2015). Furthermore, when Nestin+ cells when transplanted into matrigel in a wild-type host, they were found to have a mural cell potential within the newly

formed WAT, strongly associated with expression of PDGFRα, opposing adipogenesis (Iwayama et al., 2015).

We further observed that some Nestin+ cells were not associated with blood vessels (CD31+), although they were a minority percentage (Fig 6 C). Previous studies of our group observed that Nestin-GPF+ cells derived from the blood circulation, not associated to blood vessels, that co-express markers suggestive of hematopoietic cells, expressing Sca-1, CD45 and CXCR4 (Coimbra-Campos et al., 2021). To assess whether Nestin+ cells could derive from the circulation and be recruited due to 2-week cold exposure, we perform parabiosis of WT mice with NG2-DsRed+/Nestin-GFP+ mice, and we observed a similar characterization to our initial experiment (Fig. 1 and Fig.7).

After analyzed the adipose tissue of the mice without endogenous fluorescence, WT, we observed that for iWATp tissue, there was a considerable amount of Nestin+ cells, whereas for the iWATd tissue, these cells were present in a lower percentage. We suggest that the cells present in iWATp tissue may be present due to a cell-cell effect on migration, as first described in the classical studies by Abercrombie (Abercrombie & Heaysman, 1954). The process of cell migration is necessary for some physiological processes such as biological development and also in wound repair, pathological processes such as metastasis and cancer (Li & Wang, 2018). Since adipose tissue undergoes intense remodeling upon cold exposure, we suggest that the pericytes could have migrated into the iWATp. However, a better understanding of the contact-mediated responses of cell migration is still needed.

In addition, in the BAT, we saw possible Nestin-GFP+ structures, similar to nerve fiber bundles (NFBs), describe by (Stavely et al., 2022). Recently it has been shown that adipose tissue that is innervated by the sympathetic nervous system may be a better source of neuronal precursor cells. This is because Jumabay et al., (Jumabay, Zhang, Yao, & Bostrom, 2022) observed that neuron-like cells (GFAP+, Nestin+,βTubIII) are able to derive from adipose cells that express adiponectin . However, investigating whether Nestin+ cells present in different tissues could migrate to adipose tissue and thus increase the capacity for neurogenesis, with consequent improvement in the ability to produce thermogenesis non-shivering, still remains a lack in the literature.

In conclusion (Fig. 9), our study demonstrated that 2-week cold exposure is effective in increasing vascularization, and pericytes. However, we observed that although type-1 pericytes increase in all adipose tissues evaluated, only type-2 pericyte increases have tissues that are more susceptible to phenotypic changes after cold

exposure. We also observed that Nestin+-only cells can appear in a smaller proportion, detached from the blood vessels. Our data suggest that these cells may derive from the blood circulation. However, further analyses and data should reinforce these findings. Lineage tracing studies should also be done to better elucidate the potential for pericytes to contribute to the phenotypic changes in adipose tissue following cold stimulation. Our data open up new potential for manipulating pericytes in therapeutic strategies.

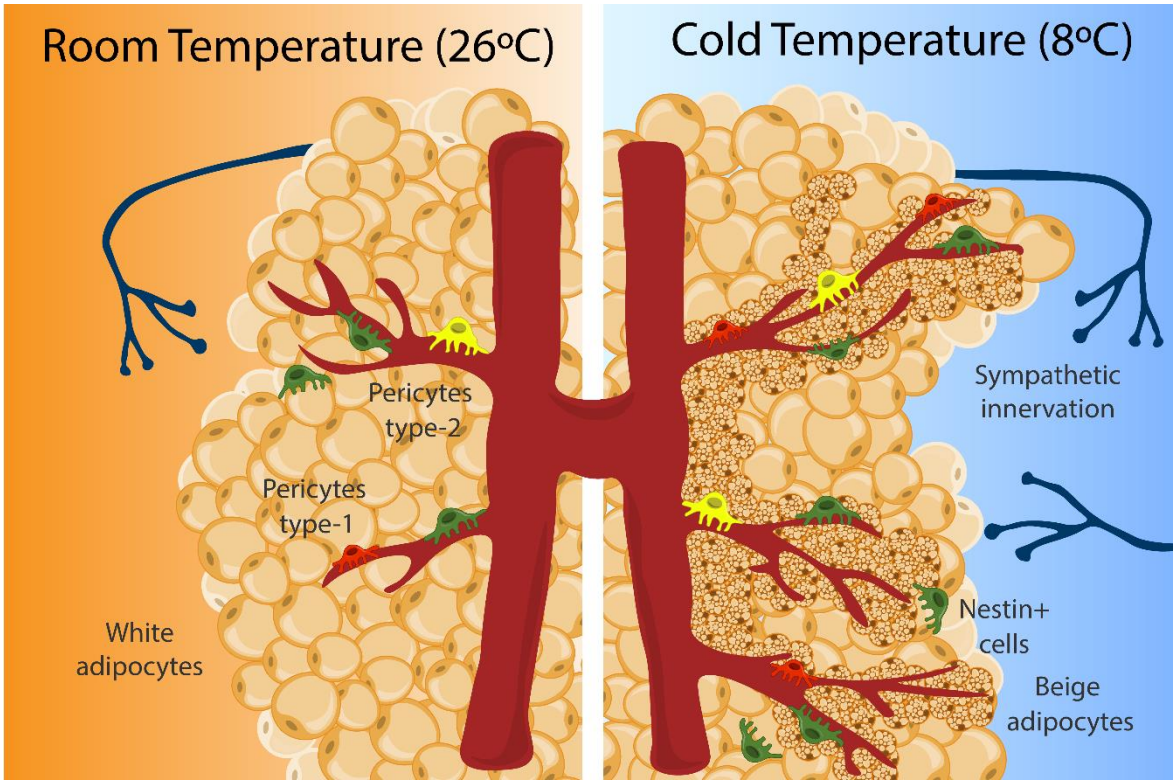


Figure 9 - Representative schematic of the microenvironment of white adipose tissue under conditions of 26°C (Room temperature) and 8°C (Cold temperature) exposure for two weeks showed that there was an increase in type 1 and 2 pericytes.

4. SCIENTIFIC PRODUCTION

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REVIEW

Pericytes Act as Key Players in Spinal Cord Injury

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Spinal cord injury results in locomotor impairment attributable to the formation of an inhibitory fibrous scar, which prevents axonal regeneration after trauma. The scarcity of knowledge about the molecular and cellular mechanisms involved in scar formation after spinal cord lesion impede the design of effective therapies. Recent studies, by using state-of-the-art technologies, including genetic tracking and blockage of pericytes in combination with optogenetics, reveal that pericyte blockage facilitates axonal regeneration and neuronal integration into the local neural circuitry. Strikingly, a pericyte subset is essential during scarring after spinal cord injury, and its arrest results in motor performance improvement. The arising knowledge from current research will contribute to novel approaches to develop therapies for spinal cord injury. We review novel advances in our understanding of pericyte biology in the spinal cord. *(Am J Pathol 2019, 189: 1327–1337; https://doi.org/10.1016/j.ajpath.2019.03.008)*

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Review

Neural stem cell niche heterogeneity

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HIGHLIGHTS

- Neural stem cells are not equally plastic homogeneous cells, but rather a combination of distinct subpopulations.
- The cerebrospinal fluid is an essential component of the neural stem cell niche.
- Neural stem cells auto-regulate themselves.
- Innervations release neurotransmitters to neural stem cells, and affect neural stem cell behavior.
- The biggest challenge remains to study the neural stem cell niche in humans.

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ABSTRACT

In mammals, new neurons can be generated from neural stem cells in specific regions of the adult brain. Neural stem cells are characterized by their abilities to differentiate into all neural lineages and to self-renew. The specific microenvironments regulating neural stem cells, commonly referred to as neurogenic niches, comprise multiple cell populations whose precise contributions are under active current exploration. Understanding the cross-talk between neural stem cells and their niche components is essential for the development of therapies against neurological disorders in which neural stem cells function is altered. In this review, we describe and discuss recent studies that identified novel components in the neural stem cell niche. These discoveries bring new concepts to the field. Here, we evaluate these recent advances that change our understanding of the neural stem cell niche heterogeneity and its influence on neural stem cell function.

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TISSUE-SPECIFIC PROGENITOR AND STEM CELLS

STEM CELLS TRANSLATIONAL MEDICINE

Sensory nerves in the spotlight of the stem cell niche

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Abstract

Niches are specialized tissue microenvironments which control stem cells functioning. The bone marrow mesenchymal stem cell niche defines a location within the marrow in which mesenchymal stem cells are retained and produce new cells throughout life. Deciphering the signaling mechanisms by which the niche regulates stem cell fate will facilitate the use of these cells for therapy. Recent studies, by using state-of-the-art methodologies, including sophisticated in vivo inducible genetic techniques, such as lineage-tracing Cre/loxP mediated systems, in combination with pharmacological inhibition, provide evidence that sensory neuron is an important component of the bone marrow mesenchymal stem cell niche. Strikingly, knockout of a specific receptor in sensory neurons blocked stem cell function in the bone marrow. The knowledge arising from these discoveries will be crucial for stem cell manipulation in the future. Here, we review recent progress in our understanding of sensory nerves biology in the stem cell niche.

KEYWORDS
genetic depletion, mesenchymal stem cells, microenvironment, niche, sensory nerves

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ORIGINAL ARTICLE

WILEY

Ablation of sensory nerves favours melanoma progression

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Abstract

The tumour mass is composed not only of heterogeneous neoplastic cells, but also a variety of other components that may affect cancer cells behaviour. The lack of detailed knowledge about all the constituents of the tumour microenvironment restricts the design of effective treatments. Nerves have been reported to contribute to the growth and maintenance of numerous tissues. The effects of sensory innervations on tumour growth remain unclear. Here, by using state-of-the-art techniques, including Cre/loxP technologies, confocal microscopy, in vivo-tracing and chemical denervation, we revealed the presence of sensory nerves infiltrating within the melanoma microenvironment, and affecting cancer progression. Strikingly, melanoma growth in vivo was accelerated following genetic ablation or chemical denervation of sensory nerves. In humans, a retrospective analysis of melanoma patients revealed that increased expression of genes related to sensory nerves in tumours was associated with better clinical outcomes. These findings suggest that sensory innervations counteract melanoma progression. The emerging knowledge from this research provides a novel target in the tumour microenvironment for therapeutic benefit in cancer patients.

KEYWORDS
genetic depletion, melanoma, sensory nerves, tumour microenvironment

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RESEARCH ARTICLE

Resistance exercise training induces subcutaneous and visceral adipose tissue browning in Swiss mice

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Picoli CD, Gilio GR, Henriques F, Leal LG, Besson JC, Lopes MA, Franzói de Moraes SM, Hernandes L, Batista Junior ML, Peres SB. Resistance exercise training induces subcutaneous and visceral adipose tissue browning in Swiss mice. *J Appl Physiol* 129: 66–74, 2020. First published June 5, 2020; doi:10.1152/japplphysiol.00742.2019.—Aerobic exercise training (AER) may promote several adaptations in white adipose tissue (WAT), including a phenotypic change known as browning. The present study aimed at assessing if resistance exercise training (RES) would be as efficient as AER in inducing a brown-like adipocyte reprogramming in WAT. Thirty Swiss male mice were randomly divided into 3 groups with 10 animals each: *1*) sedentary (SED), *2*) AER, and *3*) RES. After the adaptation training, an incremental test was performed at the beginning of each week to adjust training load. Mice were submitted to 8 wk of AER or RES. After the experimental period, inguinal and retroperitoneal WAT (iWAT and rpWAT) and brown adipose tissue (BAT) were collected. The prescription of AER and RES was effective in increasing the performance of both groups. Also, RES presented a lower body weight than AER/SED. AER and RES reduced the area of iWAT and rpWAT adipocytes and the lipid area of BAT, induced an increase of vascular endothelial growth factor (VEGF) and cluster of differentiation 31 (CD31) and uncoupling protein 1 (UCP-1), and increased the expression of selective genes of brown and beige phenotype in adipocytes after 8 wk. In general, we demonstrated here that AER and RES training similarly induced the browning of iWAT and rpWAT.

NEW & NOTEWORTHY Aerobic exercise training (AER) induces the browning of white adipose tissue, turning adipocytes multilocular, highly vascularized and expressing uncoupling protein 1 (UCP-1). The current study compared the efficiency of resistance to aerobic exercise training to promote a brown-like phenotype. Our results suggest that both types of training similarly induce subcutaneous and visceral adipose tissue browning.

adipose tissue; beige adipocyte; exercise; UCP-1

INTRODUCTION

White adipose tissue (WAT) is an important modulator of energy metabolism since it is the main source of energy storage in the form of triglycerides in the body. In addition, it exerts a relevant endocrine role as it is responsible for the synthesis and release of various adipocytokines (66, 68). The increase in WAT mass leads to obesity, a public health problem affecting around 600 million adults worldwide (43) and associated with the emergence of various comorbidities and metabolic imbalances (41). Aerobic exercise training (AER) (44) appears in this context as a strategy not only for maintaining and/or reducing body mass but also for its great ability to improve glucose homeostasis and increase lipolysis and oxidative capacity of adipocytes (20, 27, 62, 65).

Pioneering work by Stallknecht almost three decades ago revealed that aerobic training increases mitochondrial enzyme activity in WAT, as seen in skeletal muscle (60). In addition, AER stimulates WAT remodeling, making white adipocytes multilocular, highly vascularized, expressing the uncoupling protein 1 (UCP-1) and with higher oxidative capacity (4), a phenotype similar to brown adipocytes (4, 10, 12, 39, 63, 72, 73), known as "browning" (12). Moreover, a subset population of adipocytes is found in WAT termed "beige" (26) or "brite," which presents an intermediary phenotype between white and brown adipocytes (49). These cells possess a different embryonic origin from white and brown adipocytes, expressing enriched markers such as tumor necrosis factor receptor superfamily, member 9 (*Cd137/Tnfrs9*), T-box 1 (*Tbx1*), and transmembrane protein 26 (*Tmem26*) (72).

Although several metabolic adaptations caused by AER are essential, little is known regarding the role played by resistance exercise training (RES) in weight loss, obesity prevention and WAT remodeling (51). Therefore, given the growing interest of exercise training as a regulator of adipose tissue and its possible role in inducing adipose tissue browning (61, 62, 67), we hypothesized and tested the idea that RES is capable of inducing the brown-like process in both inguinal and retroperitoneal WAT (iWAT and rpWAT).

MATERIALS AND METHODS

Animals and Experimental Design

Thirty Swiss male mice, 45 days old, were maintained in individual polypropylene cages, with bedding cleaned weekly, in an automated room for photoperiod control (12:12-h light-dark cycle) with housing temperature at 23°C. The mice were allowed to feed (Nuvilab Cr1)

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Circulating Nestin-GFP$^+$ Cells Participate in the Pathogenesis of *Paracoccidioides brasiliensis* in the Lungs

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Accepted: 6 May 2021

Abstract
Multiple infectious diseases lead to impaired lung function. Revealing the cellular mechanisms involved in this impairment is crucial for the understanding of how the lungs shift from a physiologic to a pathologic state in each specific condition. In this context, we explored the pathogenesis of Paracoccidioidomycosis, which affects pulmonary functioning. The presence of cells expressing Nestin-GFP has been reported in different tissues, and their roles as tissue-specific progenitors have been stablished in particular organs. Here, we explored how Nestin-GFP$^+$ cells are affected after lung infection by *Paracoccidioides brasiliensis*, a model of lung granulomatous inflammation with fibrotic outcome. We used Nestin-GFP transgenic mice, parabiosis surgery, confocal microscopy and flow cytometry to investigate the participation of Nestin-GFP$^+$ cells in *Paracoccidioides brasiliensis* pathogenesis. We revealed that these cells increase in the lungs post-*Paracoccidioides brasiliensis* infection, accumulating around granulomas. This increase was due mainly to Nestin-GPF$^+$ cells derived from the blood circulation, not associated to blood vessels, that co-express markers suggestive of hematopoietic cells (Sca-1, CD45 and CXCR4). Therefore, our findings suggest that circulating Nestin-GFP$^+$ cells participate in the *Paracoccidioides brasiliensis* pathogenesis in the lungs.

Keywords Nestin-GFP · Circulating cells · Lung infection · *Paracoccidioides brasiliensis*

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ORIGINAL PAPER

C(3)1-TAg in C57BL/6 J background as a model to study mammary tumor development

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Accepted: 9 May 2021

Abstract
Diagnosis and prognosis of breast cancer is based on disease staging identified through histopathological and molecular biology techniques. Animal models are used to gain mechanistic insights into the development of breast cancer. C(3)1-TAg is a genetically engineered mouse model that develops mammary cancer. However, carcinogenesis caused by this transgene was characterized in the Friend Virus B (FVB) background. As most genetic studies are done in mice with C57BL/6 J background, we aimed to define the histological alterations in C3(1)-TAg C57BL/6 J animals. Our results showed that C3(1)-TAg animals with C57BL/6 J background develop solid-basaloid adenoid cystic carcinomas with increased fibrosis, decreased area of adipocytes, and a high proliferative index, which are triple-negative for progesterone, estrogen, and human epidermal growth factor receptor 2 (HER2) receptors. Our results also revealed that tumor development is slower in the C57BL/6 J background when compared with the FVB strain, providing a better model to study the different stages in breast cancer progression.

Keywords Mammary gland · Genetically engineered mouse model · Breast cancer · Tumor development

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Hematopoietic stem cell stretches and moves in its bone marrow niche

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ABSTRACT

Hematopoietic stem cells are the most illustrious inhabitants of the bone marrow. Direct visualization of endogenous hematopoietic stem cells in this niche is essential to study their functions. Until recently this was not possible in live animals. Recent studies, using state-of-the-art technologies, including sophisticated in vivo inducible genetic approaches in combination with two-photon laser scanning microscopy, allow the follow-up of endogenous hematopoietic stem cells' behavior in their habitat. Strikingly, the new findings reveal that quiescent hematopoietic stem cells are more mobile than previously thought, and link their retained steady state within the niche to a mobile behavior. The arising knowledge from this research will be critical for the therapy of several hematological diseases. Here, we review recent progress in our understanding of hematopoietic stem cell biology in their niches.

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REVIEW

Sympathetic nerve-adipocyte interactions in response to acute stress

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Abstract
Psychological stress predisposes our body to several disorders. Understanding the cellular and molecular mechanisms involved in the physiological responses to psychological stress is essential for the success of therapeutic applications. New studies show, by using in vivo inducible Cre/loxP-mediated approaches in combination with pharmacological blockage, that sympathetic nerves, activated by psychological stress, induce brown adipocytes to produce IL-6. Strikingly, this cytokine promotes gluconeogenesis in hepatocytes, that results in the decline of tolerance to inflammatory organ damage. The comprehension arising from this research will be crucial for the handling of many inflammatory diseases. Here, we review recent advances in our comprehension of the sympathetic nerve-adipocyte axis in the tissue microenvironment.

Keywords Sympathetic nerves · IL-6 · Adipocytes · Microenvironment · Hepatocytes

Introduction

Stressful psychological circumstances are frequent in our daily life. The "fight or flight response" is presently well-defined as an evolutionary conserved physiological reaction

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Review

Pericytes cross-talks within the tumor microenvironment

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ABSTRACT

Cancer cells are embedded within the tumor microenvironment and interact dynamically with its components during tumor progression. Understanding the molecular mechanisms by which the tumor microenvironment components communicate is crucial for the success of therapeutic applications. Recent studies show, by using state-of-the-art technologies, including sophisticated *in vivo* inducible Cre/loxP mediated systems and CRISPR-Cas9 gene editing, that pericytes communicate with cancer cells. The arising knowledge on cross-talks within the tumor microenvironment will be essential for the development of new therapies against cancer. Here, we review recent progress in our understanding of pericytes roles within tumors.

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RESEARCH Open Access

Chemogenetic modulation of sensory neurons reveals their regulating role in melanoma progression

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Abstract

Sensory neurons have recently emerged as components of the tumor microenvironment. Nevertheless, whether sensory neuronal activity is important for tumor progression remains unknown. Here we used Designer Receptors Exclusively Activated by a Designer Drug (DREADD) technology to inhibit or activate sensory neurons' firing within the melanoma tumor. Melanoma growth and angiogenesis were accelerated following inhibition of sensory neurons' activity and were reduced following overstimulation of these neurons. Sensory neuron-specific overactivation also induced a boost in the immune surveillance by increasing tumor-infiltrating anti-tumor lymphocytes, while reducing immune-suppressor cells. In humans, a retrospective in silico analysis of melanoma biopsies revealed that increased expression of sensory neurons-related genes within melanoma was associated with improved survival. These findings suggest that sensory innervations regulate melanoma progression, indicating that manipulation of sensory neurons' activity may provide a valuable tool to improve melanoma patients' outcomes.

Keywords: Sensory neurons, Tumor microenvironment, Melanoma, Neuronal activity, Chemogenetics

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ORIGINAL PAPER

Tissue-resident glial cells associate with tumoral vasculature and promote cancer progression

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Abstract
Cancer cells are embedded within the tissue and interact dynamically with its components during cancer progression. Understanding the contribution of cellular components within the tumor microenvironment is crucial for the success of therapeutic applications. Here, we reveal the presence of perivascular GFAP+/Plp1+ cells within the tumor microenvironment. Using in vivo inducible Cre/loxP mediated systems, we demonstrated that these cells derive from tissue-resident Schwann cells. Genetic ablation of endogenous Schwann cells slowed down tumor growth and angiogenesis. Schwann cell-specific depletion also induced a boost in the immune surveillance by increasing tumor-infiltrating anti-tumor lymphocytes, while reducing immune-suppressor cells. In humans, a retrospective in silico analysis of tumor biopsies revealed that increased expression of Schwann cell-related genes within melanoma was associated with improved survival. Collectively, our study suggests that Schwann cells regulate tumor progression, indicating that manipulation of Schwann cells may provide a valuable tool to improve cancer patients' outcomes.

Keywords Tumor microenvironment · Perivascular cells · Glia · Genetic depletion

Beatriz G. S. Rocha, Caroline C. Picoli and Bryan O. P. Gonçalves are co-first authors.

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7. APPENDIX

UFMG

UNIVERSIDADE FEDERAL DE MINAS GERAIS

CEUA
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

Prezado(a):

Esta é uma mensagem automática do sistema Solicite CEUA que indica mudança na situação de uma solicitação.

Protocolo CEUA: 15/2022
Título do projeto: PAPEL DAS CÉLULAS NG2+/NESTIN+ NO TECIDO ADIPOSO DE CAMUNDONGOS SUBMETIDO A EXPOSIÇÃO AO FRIO
Finalidade: Pesquisa
Pesquisador responsável: Alexander Birbrair
Unidade: Instituto de Ciencias Biologicas
Departamento: Departamento de Patologia

Situação atual: Decisão Final - Aprovado

Aprovado na reunião extraordinária on-line do dia 04/04/2022. Validade: 04/04/2022 à 03/04/2027.

Belo Horizonte, 04/04/2022.

Atenciosamente,

Sistema Solicite CEUA UFMG
https://aplicativos.ufmg.br/solicite_ceua/

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