MUSCULOSKELETAL PATHOLOGY

Characterization of Brown Adipose—Like Tissue in Trauma-Induced Heterotopic Ossification in Humans

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Heterotopic ossification (HO), the abnormal formation of bone within soft tissues, is a major complication after severe trauma or amputation. Transient brown adipocytes have been shown to be a critical regulator of this process in a mouse model of HO. In this study, we evaluated the presence of brown fat within human HO lesions. Most of the excised tissue samples displayed histological characteristics of bone, fibroproliferative cells, blood vessels, and adipose tissue. Immunohistochemical analysis revealed extensive expression of uncoupling protein 1 (UCP1), a definitive marker of brown adipocytes, within HO-containing tissues but not normal tissues. As seen in the brown adipocytes observed during HO in the mouse, these UCP1⁺ cells also expressed the peroxisome proliferator-activated receptor γ coactivator 1 α . However, further characterization showed these cells, like their mouse counterparts, did not express PR domain containing protein 16, a key factor present in brown adipocytes found in depots. Nor did they express factors present in beige adipocytes. These results identify a population of UCP1⁺ cells within human tissue undergoing HO that do not entirely resemble either classic brown or beige adipocytes, but rather a specialized form of brown adipocyte-like cells, which have a unique function. These cells may offer a new target to prevent this unwanted bone. (*Am J Pathol* 2017, 187: 2071–2079; <http://dx.doi.org/10.1016/j.ajpath.2017.05.012>)

Heterotopic ossification (HO) is the ectopic formation of bone in soft tissues. HO most frequently occurs after traumatic injury to the brain and spinal cord,¹ burns,² fractures,³ and blasts.^{4,5} Within the military, the prevalence of HO has been shown to be 64% in soldiers experiencing severe combat-related injuries.⁶ In some instances, this ectopic bone formation can surround nerves and vessels, resulting in their dysfunction.⁷ This can occur in addition to ulcerations, pain, and limited joint motion, which are more common complications of HO. Currently, the primary means of treatment for persistently symptomatic HO involves surgical excision of the bony lesion.⁸ The removal of these lesions can be problematic, requiring precise excision as well as wound complications. Furthermore, in certain cases, the lesions can recur. Therefore, the ability to detect and/or potentially block the progression of HO during its earliest stages would greatly improve efforts to prevent and treat this aberrant process.

Our previous studies, using a mouse model where HO is induced by bone morphogenetic protein 2, found transient brown adipocytes drive the earliest steps of HO by generating a microenvironment conducive to bone formation.^{9–11} These cells are defined by their expression of uncoupling protein 1 (UCP1). UCP1 reduces the concentration of oxygen in the microenvironment, whereas the standard ATPase does not, because UCP1 has a much higher catalytic activity (nmole O min⁻¹ mg mitochondrial protein⁻¹) at a given membrane potential.¹² It is this higher activity coupled with its ability to

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generate heat, because oxygen solubility decreases as temperature increases, that causes UCP1 to function as a generator of a hypoxic microenvironment. It is one of the few proteins that can generate hypoxia, and we have previously shown that it can effectively accomplish this.⁹ Characterization of this transient brown adipose tissue (tBAT) induced during HO in mice revealed its cells selectively express many of the factors previously implicated in classic brown adipocyte development and function, such as the transcriptional regulator peroxisome proliferator-activated receptor γ coactivator 1 α .^{9,11} However, these cells differ from classic brown adipocytes found in dedicated depots in that they do not express PRDM16,¹¹ which has been shown to regulate the differentiation of myoblastic precursors into brown adipose tissue (BAT).¹³ These studies, therefore, suggest a specialized pool of brown adipocyte-like cells that contribute to HO.

In our mouse model of HO, we found that the presence of tBAT coincides with the presence of hypoxia and the ultimate deposition of cartilage.⁹ The tBAT forms a region that is flanked on one side by cartilage (hypoxia) and on the other side by vessel formation (normoxia).^{9,11} This vessel formation is stimulated by the secretion of angiogenic factors from tBAT.¹⁰ Therefore, tBAT is involved in the patterning of bone and cartilage and is more than a simple generator of heat.

In recent years, the presence of BAT within adult humans has been found to be more widespread than originally thought.^{14–18} Depots of UCP1⁺ brown fat have been identified in specific regions throughout the body (supraclavicular, cervical, perirenal).^{15,16,19,20} Furthermore, cells with a brown fat-like phenotype that express UCP1, referred to as brite or beige adipocytes, can be induced within white fat depots.^{21,22} In rodents, this has been shown to occur on exposure to cold or β 3-adrenergic stimulation. Sidossis and Kajimura²³ recently confirmed the emergence of UCP1⁺ brown fat-like cells within subcutaneous white fat after the prolonged adrenergic stress experienced by burn patients.²⁴ Interestingly, brown adipocytes associated with HO in mice have been shown to express the β 3-adrenergic receptor (ADRB3) and to proliferate in response to noradrenaline.¹¹ Because HO is often associated with severe burns, these cell populations may be similar.

Given the critical role identified for brown adipocyte-like cells in patterning HO in mice,⁹ as well as their emerging role in tissue regeneration, as evidenced by the extreme osteoporotic phenotype of mice lacking a key component in the formation of tBAT,²⁵ we sought to determine if a similar cell could be found within human HO tissues.

Materials and Methods

Tissue Sample Collection

Human tissues were obtained during surgical removal of symptomatic lesions from 24 different patients experiencing a combat-related trauma to the extremities at Walter Reed National Military Medical Center, through an approved

Walter Reed Medical Center institutional review board protocol (number 374863). All human tissue transfers to Baylor College of Medicine (E.A.O.-D.) under an approved institutional review board protocol (H-30833) from Walter Reed National Military Medical Center (J.A.F.) followed the approved Cooperative Research and Development Agreement (NCRADA-NMRC-13-9127) between Baylor College of Medicine and the Department of the Navy.

These samples were classified as either early, preradiographic HO with palpable evidence of mineralization ($n = 20$) or mature HO with radiographically apparent ectopic bone formation ($n = 4$). Other properties of the patient population have been described previously.^{26,27} Control samples were obtained from discarded tissue collected from extremity-injured patients during an initial wound debridement procedure, before final wound closure. Subsequent follow-up of these patients confirmed none of them developed radiographic evidence of HO. All collected tissues were fixed in 10% neutral-buffered formalin for histological analysis.

Histological Assessment

Tissues were fixed and transferred in 10% neutral-buffered formalin, decalcified in Richard-Allan Scientific Cal-Rite Decalcifying Solution (ThermoScientific, Kalamazoo, MI), trimmed for mounting in cassettes, and then processed and paraffin embedded. Serial sections (5 μ m thick) were prepared, and every 10th section throughout the tissue sample was stained with hematoxylin and eosin for histological analysis. The tissue sections were examined by two authors (E.A.S. and A.R.D.), who were blinded to the tissue collection and radiographic results, for the following histological features: bone, fibroproliferation, blood vessels, nerve, cartilage, mature muscle, and adipose tissue (both white and brown adipocytes).

Bone was characterized by an eosinophilic matrix deposition, which was often accompanied by osteoblasts, osteoclasts, or osteocytes. Fibroproliferative lesions were identified as areas of accumulated fibroblastic cells rich with collagen fibers and other matrix components. Blood vessels were defined as thin vascular spaces, and nerves were identified by their characteristic arrangement of nerve fibers. Cartilage was classified by chondrocytes surrounded by a lacunar space and abundant matrix. Adipose tissue was identified as cells with globules of lipid-like material filling the cytoplasm. White fat is distinguished as a single large globule with a thin ring of cytoplasm and eccentric nuclei, whereas brown fat is differentiated by its multiple lipid droplets and abundant mitochondria, giving the appearance of a darker cell with a soap-bubble cytoplasm.

Immunohistochemical Analysis

Serial unstained slides, adjacent to hematoxylin and eosin-stained sections, were used for immunohistochemical staining (either single- or double-antibody labeling), using

Table 1 Histological Assessment of Heterotopic Ossification Tissue Samples

Histological feature	Presence, <i>n</i> (%)
Bone	22 (92)
Fibroproliferative lesion	17 (71)
Cartilage	5 (21)
Nerve	3 (12.5)
Blood vessels	13 (54)
White/brown adipocytes	18 (75)
Mature muscle	16 (67)

n = 24 samples.

methods outlined previously.⁹ For double-antibody labeling, samples were treated with both primary antibodies simultaneously, followed by washing and incubation with respective secondary antibodies, used at a 1:500 dilution, to which Alexa Fluor 488 or 594 was conjugated. Primary antibodies were used as follows: UCP1, rabbit polyclonal, used at 1:100 dilution (EMD Millipore, Billerica, MA); peroxisome proliferator-activated receptor γ coactivator 1 α , goat polyclonal, used at 1:100 dilution (LifeSpan Biosciences, Seattle, WA); PRDM16, rabbit polyclonal, used at 1:100 dilution (Abcam, Cambridge, MA); T-Box 1, rabbit polyclonal, used at a 1:100 dilution (Abcam); CD137, mouse monoclonal, used at a 1:100 dilution (Abcam); peroxisome proliferator-activated receptor γ , rabbit polyclonal, used at 1:200 dilution (Abcam); Homeobox C9, mouse monoclonal, used at 1:100 dilution (Abcam); Sry-Box 9, rabbit polyclonal, used at 1:100 dilution (Santa Cruz Biotechnology, Santa Cruz, CA); aggrecan, goat polyclonal, used at 1:20 dilution (R&D Systems, Minneapolis, MN); neurofilament, mouse monoclonal, used at 1:200 dilution (Sigma-Aldrich, St. Louis, MO); and a mouse polyclonal antibody to the human ADRB3, used at 1:100 dilution (Abnova, Taipei City, Taiwan). Primary antibodies were diluted in either 2% bovine serum albumin or phosphate-buffered saline and 10% serum of the species in which the secondary antibody was generated. Tissues were mounted and counterstained using Vectashield mounting medium containing DAPI (Vector Laboratories, Burlingame, CA). Stained tissue sections were examined using an Olympus BX41 microscope (Olympus Corporation of the Americas, Waltham, MA) equipped with a reflected fluorescence system, using a 10 $\times$ /0.40 numerical aperture, 20 $\times$ /0.75 numerical aperture, or 40 $\times$ /0.85 numerical aperture objective lens.

Results

Histological Characterization of Tissue

A total of 24 HO tissue samples were analyzed for specific histological features (Table 1). Figure 1 shows representative tissue sections stained with hematoxylin and eosin. Bone was histologically apparent in 22 (92%) of the samples, consisting of both immature (Figure 1A) and mature (Figure 1B) bone areas. The immature HO tissue contained numerous

osteoblasts lining the bone surface and multinucleated osteoclasts, suggesting active bone remodeling. Mature HO tissue, on the other hand, was characterized by a more lamellar arrangement of the bone matrix. We observed fibroproliferation, which has been reported in other forms of HO,²⁸ in 17 (71%) (Figure 1C) and cartilage formation in 5 (21%) (Figure 1D) of the tissue specimens. Surrounding muscle tissue was noted in 16 (67%) of the HO tissues (Figure 1H). We also observed nerves and blood vessels in 3 (12.5%) and 13 (54%) of the tissue samples of HO, respectively (Figure 1, E and F). Finally, we found adipose tissue, with regions of histology typical of both white and brown fat, in 75% of the tissues analyzed (Figure 1G).

Expression of UCP1 within HO Tissue Samples

Brown adipocyte-like cells have been shown to be a central regulator of HO in mouse models because hypoxic regions produced by these cells are essential for chondrogenesis.^{9,11}

Figure 1 Histological assessment of HO tissue. Representative images of tissue sections from HO samples stained with hematoxylin and eosin. Photomicrographs of histological features identified within collected HO tissues, including immature (A) and mature (B) bone, fibroproliferative tissue (C), cartilage (D), nerves (E), blood vessels (asterisks; F), adipose tissue (G), and the surrounding muscle fibers (H). Scale bars: 100 μ m (A–C and E–H); 50 μ m (D).

Furthermore, adjoining regions characterized by vessel formation, which brown adipocyte-like cells also regulate,^{10,11} serve as osteoprogenitor entry points via extravasation.²⁹ We assessed expression of the brown adipocyte-specific marker UCP1 within the tissue samples of human HO by immunohistochemistry. Figure 2 contains representative images of the UCP1 expression observed within different tissue samples. Hematoxylin and eosin staining was used. UCP1⁺ cells were found within regions of adipose tissue adjacent to bone (Figure 2), muscle fibers (Figure 2), and vessels (Figure 2). UCP1⁺ cells were found within approximately 63% (15 of 24) of the human HO tissues analyzed. There was no detectable UCP1 expression in control tissues from patients who did not develop HO (Figure 3).

UCP1⁺ Adipocytes in HO Tissues Express the Key Transcription Factor Peroxisome Proliferator-Activated Receptor γ Coactivator 1 α , but Not PDRM16 or Markers of Beige Fat Cells

To further characterize the UCP1⁺ adipocytes observed in the HO tissues, we immunostained for markers reported to be present in brown and beige adipocytes.²¹ Regions of adipocytes were identified by expression of the transcriptional regulator peroxisome proliferator-activated receptor γ (Figure 4E). Serial tissue sections were coimmunostained with UCP1 and peroxisome proliferator-activated receptor γ coactivator 1 α , which interacts with peroxisome proliferator-activated receptor γ and is preferentially expressed in brown/beige adipocytes as compared to white adipocytes,³⁰ and we observed colocalization of these factors (Figure 4, A–D). In contrast, UCP1⁺ cells were negative for expression of PRDM16 (Figure 4F) and the beige fat markers CD137 (Figure 4G), T-Box 1 (Figure 4H), and Homeobox C9 (data not shown).²¹

UCP1⁺ Cells within Cartilage and Nerves in HO Tissues

Immunohistochemical staining for UCP1 also revealed cells positive for this protein within unique regions of the tissue samples of HO, including areas of cartilage and nerves. UCP1 expression (Figure 5B) was observed within cartilage, which was identified by staining with the cartilage marker aggrecan³¹ (Figure 5C) and the precartilaginous marker Sry-Box 9³² (Figure 5D). Cells positive for UCP1 (Figure 5G) were also found in peripheral nerves within the HO tissues, as demonstrated by neurofilament staining (Figure 5H). Hematoxylin and eosin staining (Figure 5, A and F) and merged views (Figure 5, E and I) were also used. UCP1 expression was not detected in these regions within control tissues that were not undergoing HO (data not shown).

Expression of ADRB3 in HO Tissue

The expression of ADRB3 was assessed in HO tissue along with UCP1 (Figure 6). We observed various patterns of

Figure 2 Analysis of UCP1 expression within HO tissue samples. **A, C, E, G, I, and K:** Corresponding serial sections stained with hematoxylin and eosin (H&E). **B, D, F, H, J, and L:** Representative photomicrographs of HO tissues immunostained with UCP1 (green). DAPI (blue) was used as a counterstain to identify cell nuclei (**B**). These regions of UCP1⁺ fat are found interspersed with bone (**C** and **E**), muscle (**G**), and vessels (**K**). Scale bar = 100 μ m (**A–L**).

staining. In the first pattern, staining was mostly in the nerve, with UCP1 staining primarily in the perineurium and staining for ADRB3 in the endoneurium (Figure 6A). The second pattern showed staining for both ADRB3 and UCP1

Figure 3 Analysis of UCP1 expression within control tissue samples. Representative photomicrographs of control tissues from extremity-injured patients who did not develop evidence of HO. **A** and **C**: The corresponding serial sections stained with hematoxylin and eosin (H&E) are shown. **B** and **D**: Samples were immunostained with UCP1 and found to be negative for UCP1 staining. Tissues were counterstained with DAPI (blue) to identify cell nuclei. Representative regions of fat and muscle within the samples, areas found to be positive in the HO tissues, are highlighted here. Scale bars = 100 μ m (**A–D**).

within regions of adipose tissue and vessels (Figure 6B). The third pattern showed staining for both ADRB3 and UCP1 in cells lining the surface of bone (Figure 6C).

Radiographic Analysis of Heterotopic Ossification

Extremity wounds from blast-related combat injuries are typically extremely large and complex because the patients

undergo multiple rounds of surgical debridements (2 to 14 total) to remove nonviable tissue and biofilm. Also, revisions (limb salvage) are conducted before definitive wound closure at 7 to 21 days after injury. The samples analyzed herein termed early HO were taken from patients with no radiographic evidence of HO. However, X-rays and computed tomographic scans were taken routinely during postoperative care to evaluate the wound healing and to monitor for HO development. The images shown in Figure 7 illustrate the radiographic HO development that occurs 4 to 20 weeks after injury in these transfemoral amputees. No ectopic bone was observed by X-ray before 14 days. At 4 weeks, patients developed wispy evidence of tissue mineralization that progressed to form isolated islands of bone in the soft tissues and exostoses that extend from the distal end of the residual femur into the soft tissue. The HO continued to mature over 6 to 20 weeks. Representative X-ray analyses of two patients at 4 (Figure 7A) and 20 (Figure 7B) weeks after injury are shown in Figure 7.

Discussion

The brown fat that appears in human HO lesions can be characterized by comparison with known phenotypes of brown adipose tissue found naturally in the human body. Most of the adipose tissue observed in our study expressed UCP1, confirming that it is similar to classic brown fat in this important aspect and indicating that the tissue is capable of thermogenesis through uncoupled aerobic respiration. Furthermore, the data indicate that the UCP1⁺ cells observed most closely resembled brown fat as opposed to

Figure 4 Characterization of UCP1⁺ adipocytes within HO tissues. **A**: Image of the same region stained with hematoxylin and eosin (H&E) on a tissue section adjacent to the sections used for immunostaining. **B** and **C**: Representative images of UCP1 expression in the HO tissues shows UCP1⁺ cells (green; **B**) coexpressing peroxisome proliferator-activated receptor γ coactivator 1 α (PGC1 α ; red; **C**). **D**: A merged image of **B** and **C**. **E**: Peroxisome proliferator-activated receptor γ (PPAR γ) expression (red) is observed colocalizing with these UCP1-expressing cells. **F–H**: Additional immunostaining of serial sections reveals that these cells do not express the transcriptional regulator PRDM16 (**F**), or typical markers of beige fat, including CD137 (**G**) and T-Box 1 (TBX1) (**H**). A small amount of autofluorescence from red blood cells was noted within these sections. Scale bar = 100 μ m (**A–H**).

Figure 5 Cartilage and nerves contain cells positive for UCP1. Representative images of areas of cartilage and nerve within the HO tissue found to express UCP1. **A** and **F**: Adjacent hematoxylin and eosin (H&E)-stained sections from the same tissue are shown. **B** and **G**: Tissues were serially sectioned, immunostained with an antibody to UCP1 (green), and analyzed. **C** and **D**: Cartilage tissue was identified by coimmunostaining of a serial section with antibodies to both aggrecan (green; **C**) and the nuclear transcription factor Sry-Box 9 (Sox9; red; **D**). A subset of the aggrecan-positive cells coexpressed Sox9, which is an earlier marker of cartilage formation and indicative of chondrogenesis. **E**: A merged image of **C** and **D**. **H**: Nerves were identified by the neural marker neurofilament (NF; red). **I**: A merged image of **G** and **H**. Scale bars = 100 μ m (**A**–**I**).

beige fat, as they were negative for CD137, T-Box 1, and Homeobox C9, all markers for the beige/brite fat subtype.²³ However, the data demonstrate a lack of expression of PRDM16, indicating that these cells have an origin different from the brown fat they resemble. Brown fat contained in depots arises from precursors that express Myf5,¹³ and its biogenesis requires the expression of PRDM16, a transcription factor essential for the differentiation of precursors into brown fat instead of muscle.¹³ In our previous studies using our mouse model, induction of HO caused the biogenesis of similar brown adipocyte-like cells, but these cells also did not express PRDM16.¹¹ In the mouse model, perineurial cells expressing ADRB3 that lined peripheral nerves not only migrated toward the site of HO but also expressed angiogenic factors.^{10,11} On activation of ADRB3, mitochondrial synthesis in these cells increased and their expression of UCP1 increased 70-fold, leading to their brown adipocyte-like appearance.^{9,11} We, therefore, suspect that these cells phenotypically resemble brown more closely than beige fat, yet bear unique characteristics and origin.

It is also noteworthy that cells expressing UCP1 described herein seem to be present both in sections where there is little or no bone present, as well as in sections containing either immature or mature bone. Surprisingly, the UCP1⁺ cells also appear to surround the bone in many instances. Although the reasons for this are unclear, we have found that tBAT in the mouse model contains osteocalcin (A.R.D. and E.A.O.-D., unpublished data) as well as citrate.³³ Because osteocalcin is the most abundant noncollagenous component of the bone matrix, whereas citrate comprises between 1% and 2% by weight of the

matrix,³⁴ it is conceivable that these cells deliver at least a portion of these components to the matrix.

Blast and burn injuries lead to high incidences of HO in humans.⁸ Previous studies have shown that metabolic changes accompany HO.^{9,33} Some of these changes likely result from the appearance of brown fat in the lesion and subsequent uncoupled aerobic respiration and altered triglyceride homeostasis.³⁵ Studies evaluating brown fat activation after burn injury indicate it contributes to the metabolic changes observed after burns.^{36,37} The induction of brown fat-mediated changes in metabolism may have a connection to HO pathology because of burn injury. Surprisingly, in all cases, the appearance of these brown fat cells in white fat have been linked to the β 3-adrenergic receptor.^{23,24}

The UCP1⁺ adipocytes seen in the human HO tissue samples in this study display a similar phenotype to the transient brown adipocytes previously identified using our mouse model of HO.^{11,38} The brown adipose-like tissue that predates ossification in both models likely has a similar role. We suspect the brown adipocyte-like cells in the human lesions function to generate a microenvironment conducive for chondrogenesis and bone formation, as we have seen in our mouse studies. The ability of tBAT to perform uncoupled aerobic respiration provides an effective means of generating a hypoxic local environment. The reduced oxygen tension stimulates angiogenesis, an essential step in the formation of bone.^{9,10} Furthermore, recent studies in tissues at risk for HO have shown that the enhancement of microcirculation, which consequently prevents local tissue hypoxia, significantly reduced the occurrence of HO.³⁹ These findings suggest that the brown

Figure 6 Costaining of ADRB3 and UCP1 in human HO tissue. Three tissue samples of human HO were stained with ADRB3 (red) and UCP1 (green). Adjacent hematoxylin and eosin (H&E)–stained sections from the same tissue are shown. Scale bar = 200 μ m (A–C).

adipocyte-like cells may contribute to providing a permissive environment for HO. The tBAT also secretes vascular endothelial growth factors A and D.¹⁰ This causes neo-vascularization at the site of bone formation, enabling osteoprogenitors from the nerve to flow to and then extravasate across the vessel wall into the site of new bone formation.²⁹

We observed UCP1⁺ cells in nerves and cartilage in the tissue samples of human HO. Our previous study of bone morphogenetic protein 2–induced HO shows that these brown adipocyte-like cells in mice come from peripheral nerve progenitor cells.¹¹ The presence of UCP1⁺ cells in peripheral nerves observed in the human HO lesions further supports those findings. The presence of UCP1⁺ cells in cartilage may reflect a common origin of transient brown fat and chondrocytes. Using lineage tracing, we recently reported a multipotential, Wnt1⁺ neural progenitor that produces both prechondrocytes and transient brown fat during HO in the mouse.²⁶

We observed three staining patterns for ADRB3 and UCP1 in HO tissues. In the pattern observed only in nerves, UCP1 was expressed primarily in cells in the perineurium, whereas ADRB3 was expressed mostly in the endoneurium. Although there was a large and significant increase in expression of ADRB3 in both the endoneurium and perineurium in the mouse model after bone morphogenetic protein 2 induction, at no time was this receptor expressed

mostly in the endoneurium. Therefore, in human HO, there was minimal overlap of the expression of ADRB3 and UCP1 in the nerve, whereas there was considerable overlap in the mouse model.¹¹ Although we do not understand the reason for this difference, there is considerable variation between the anatomy of mouse and human nerves in that human nerves have multiple fascicles as well as a much more substantial epineurium and perineurium. Outside of

Figure 7 Radiographic analysis of HO. X-rays of patients with a transfemoral amputation at 1 (A) and 5 (B) months after combat injury. White arrows indicate residual femur; red arrows, radiographically evident heterotopic bone in the soft tissue.

the nerve, there is considerable, but not complete, overlap of ADRB3 and UCP1.

In several of the HO tissue samples, we noted the presence of nerves embedded within the region of newly forming bone. The traumatic insult that induces HO in these tissues likely results in injury to the peripheral nerves as well, which may activate neural progenitors. As the neural tissue is spared during surgery, we did not detect nerves in all of the samples. However, in certain sections, there were many nerves observed where few were expected.

In this study, we translated our previous findings from our mouse model of HO to the human disease. We detected brown adipose–like tissue in human HO induced by traumatic injury with features similar to those of brown fat in the mouse model. Understanding the key cellular players contributing to HO and the specific mechanisms related to this aberrant process can help identify new targets to prevent or treat this condition.

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