様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

# 科学研究費助成事業　　研究成果報告書

令和　４ 年　 ６ 月 １７ 日現在

機関番号：１２１０２
研究種目：研究活動スタート支援
研究期間：2020～2021
課題番号：２０Ｋ２２９６９

研究課題名（和文）Study of molecular events during scarless body part regeneration in newts

研究課題名（英文）Study of molecular events during scarless body part regeneration in newts

研究代表者
ＣＡＳＣＯ．ＲＯＢＬＥＳ　Ｍａｒｔｉｎ．Ｍｉｇｕｅｌ（CASCO ROBLES, MARTIN MIGUEL）

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交付決定額（研究期間全体）：（直接経費）　　2,200,000円

研究成果の概要（和文）：成体のイモリは、傷跡なしで負傷した組織を再生することができます。本研究の成果は以下の通りです。
手肢再生:(1)Newtic-1遺伝子ノックダウン(阻害)のためのトランスジェニックプラスミドの開発は、現在テスト中です。(2)イモリ赤血球とパターニング細胞のためのin vivo細胞追跡システムの開発。
無瘢痕再生:(1)成体イモリにおける全身の様々な領域の皮膚再生能力を調査し、この動物が傷跡のない治癒のためのユニークな細胞メカニズムをもつことを示しました.(2)皮膚移植片の再配置後に四肢の再生を研究しましたが、結果は皮膚が手足のパターニングや指のパターニングに関与している可能性があります。

研究成果の学術的意義や社会的意義
In society, patients have low ability to regenerate injured tissue.  However the adult newt is capable of amazing tissue regeneration. During this startup we established in vivo tracking systems and studied the newts unique skin cells during scarless wound healing and limb regeneration.

研究成果の概要（英文）：The adult newt can regenerate injured tissue without scars. During this startup research the results were the following:
LIMB REGENERATION: (1) Development of transgenic plasmids for Newtic-1 gene knockdown (inhibit), now being tested. (2) Development of an in vivo cellular tracking systems for newt red blood cells and patterning cells.
SCARLESS SKIN: (1) We studied scarless regeneration in different skin areas of the newt, results demonstrate the newt has unique skin cells for scarless healing. (2) We studied limb regeneration following skin grafts reposition, results show that skin maybe involved in limb patterning and finger patterning.

研究分野：Regenerative Physiology

キーワード：Newt　Regeneration　Scarless　Tissue　Transgenic

## １．研究開始当初の背景

The newt (a salamander) is a classical model organism reaching back to Spallanzani. For decades the mystery of how newts regenerate injured tissue has been studied in different labs around the world. Newt tissue occurs when localized adult mature cells dedifferentiate by reprogramming into a stem-like state, which later recapitulate developmental processes. During this stem cell reprogramming (early stage of regeneration) newts evolved an anti-fibrotic mechanism to repair injured body parts. This is quite astonishing in nature and seldom seen in higher vertebrates.  In complete contrast, mammalian fibrosis is characterized with hyperproliferation, trans-differentiation of epithelial cells and fibroblasts into myofibroblasts and matrix contraction. For example, in the human eye retinal scar formation is associated with PVR (proliferative vitreoretinopathy).  Mature newt RPE (retinal pigmented epithelium) cells provide a link that similarities exist between mammalian disease and newt scar less regeneration.  Results have demonstrated a sneak peek of what happens when adult newt RPE cell lose their regenerative potential (Pax6 knockdown), it leads to a cellular fate similar to human PVR. In other words, when newts lose their regenerative potential, they express a mammalian like scar. A tentative hypothesis for this phenomena is: The newt by passes fibrosis with the aid of regenerative reprogramming genes. The key scientific/core question is: How can humans biomimic the newts scar less regeneration for medical use? Driving experimental question: How do newts regulate dedifferentiation/reprogramming of mature cells to repair tissue without fibrosis?Adult newt tissue regeneration occurs when localized mature cells dedifferentiate by reprogramming into a stem-like state, which later recapitulate developmental processes. During this stem cell reprogramming (early stage of regeneration) newts evolved an anti-fibrotic mechanism to repair injured body parts. This is quite astonishing in nature and seldom seen in higher vertebrates.  In complete contrast, mammalian fibrosis is characterized with hyperproliferation, trans-differentiation of epithelial cells and fibroblasts into myofibroblasts and matrix contraction. For example, in the human eye retinal scar formation is associated with PVR (proliferative vitreoretinopathy).  Mature newt RPE (retinal pigmented epithelium) cells provide a link that similarities exist between mammalian disease and newt scar less regeneration.  Results have demonstrated a sneak peek of what happens when adult newt RPE cell lose their regenerative potential (Pax6 knockdown), it leads to a cellular fate similar to human PVR. In other words, when newts lose their regenerative potential, they express a mammalian like scar. A tentative hypothesis for this phenomena is: The newt by passes fibrosis with the aid of regenerative reprogramming genes. The key scientific/core question is: How do newts regulate dedifferentiation/reprogramming of mature cells to repair tissue without fibrosis?

## ２．研究の目的

The purpose is to elucidate molecular events occurring during the early stages (dedifferentiation, reprogramming, and anti-fibrotic regulation) of newt tissue regeneration. Insight to these processes have scientific significance to a wide range of medical fields ranging from degenerative diseases to tissue trauma in humans. This research is unique in the sense that it aims to elucidate a natural phenomenon of mature newt cell reprogramming using a transgenic approach to seek medical applications.

## ３．研究の方法

To study skin scarring in different body part we use skin graft surgeries and transgenic newt skin expression a florescent reporter protein. Sections are later prepared for tissue sectioning. This way we can monitor the behavior of skin cells during skin regeneration. Because the role of blood and patterning is unknown in the newt we developed a transgenic reporter system for tracking blood cells, RPE cells, and Shh (patterning) gene expression in vivo in the newt. Tissue is later examined using immunohistochemistry with anti-bodies. For gene knockdown separate transgene plasmid were developed containing iRNA targeting Newtic-1 unique gene.

## ４．研究成果

During this startup the following newt transgenic lines were tested for the first time: (1) We succeeded in isolating the mouse GATA1 promoter and introducing it into the newt *Cynops pyrrhogaster*. Transgenic mGATA1 reporter cassette was expressed in F0 newt red blood cells, thus allowing future experiments to be designed to target newt red blood cells. (2) We isolated the newt and mouse ZRS (limb enhancer) for tracking cells involved in patterning during newt limb regeneration. We generated F0 transgenics newts expressing a ZRS enhancer, SHH promoter and a fluorescent reporter. Therefore, it is conceivable to track adult patterning cells participating in adult limb regeneration. This is a critical

achievement because adult humans do not re-express patterning following injury. So future experiments will also to compare human and newt patterning cell types (3) We succeeded in developing a plasmid to knock-down Newtic1 (a unique gene detected during limb regeneration) in vivo, this plasmid targets newtic1 mRNA and stops protein production via iRNA. Preliminary F0 lines are being tested. (4) Lastly, to study scarless regeneration and patterning. We studied skin grafts and transgenic skin epidermal healing, and found unique ability of newt skin cells.

5．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　2件／うちオープンアクセス　2件）

| | |
|---|---|
| 1．著者名<br>Martin Miguel Casco-Robles, Kayo Yasuda, Kensuke Yahata, Fumiaki Maruo, Chikafumi Chiba | 4．巻<br>9 |
| 2．論文標題<br>Reviewing the Effects of Skin Manipulations on Adult Newt Limb Regeneration: Implications for the Subcutaneous Origin of Axial Pattern Formation | 5．発行年<br>2021年 |
| 3．雑誌名<br>Biomedicines | 6．最初と最後の頁<br>17 |
| 掲載論文のDOI（デジタルオブジェクト識別子）<br>10.3390/biomedicines9101426 | 査読の有無<br>有 |
| オープンアクセス<br>オープンアクセスとしている（また、その予定である） | 国際共著<br>該当する |

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|---|---|
| 1．著者名<br>Atsuyuki Ishii, Ikkei Takashimizu, Martin Miguel Casco-Robles, Yuji Taya, Shunsuke Yuzuriha, Fubito Toyama, Fumiaki Maruo, Kazuo Kishi, Chikafumi Chiba | 4．巻<br>9 |
| 2．論文標題<br>Skin Wound Healing of the Adult Newt, Cynops pyrrhogaster: A Unique Re-Epithelialization and Scarless Model | 5．発行年<br>2021年 |
| 3．雑誌名<br>Biomedicines | 6．最初と最後の頁<br>12 |
| 掲載論文のDOI（デジタルオブジェクト識別子）<br>10.3390/biomedicines9121892 | 査読の有無<br>有 |
| オープンアクセス<br>オープンアクセスとしている（また、その予定である） | 国際共著<br>該当する |

〔学会発表〕　計1件（うち招待講演　1件／うち国際学会　0件）

| |
|---|
| 1．発表者名<br>Casco Robles Martin Miguel |
| 2．発表標題<br>Development of in vivo systems for studies on unique tissue patterning during adult newt regeneration |
| 3．学会等名<br>第7回イモリ型の臓器再生フォーラム（招待講演） |
| 4．発表年<br>2021年 |

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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6．研究組織

| | 氏名<br>（ローマ字氏名）<br>（研究者番号） | 所属研究機関・部局・職<br>（機関番号） | 備考 |
|---|---|---|---|

7．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

| 共同研究相手国 | 相手方研究機関 |
| --- | --- |