Healing

Li-Te Lin[a,b,c], Wen-Ling Lee[c,d,e], Peng-Hui Wang[c,f,g,h,*]

[a]Department of Obstetrics and Gynecology, Kaohsiung Veterans General Hospital, Kaohsiung,Taiwan, ROC; [b]Department of Biological Science, National Sun Yat-Sen University, Kaohsiung, Taiwan, ROC; [c]Institute of Clinical Medicine, National Yang-Ming University, Taipei, Taiwan, ROC; [d]Department of Medicine, Cheng-Hsin General Hospital, Taipei, Taiwan, ROC; [e]Department of Nursing, Oriental Institute of Technology, New Taipei City, Taiwan, ROC; [f]Department of Obstetrics and Gynecology, Taipei Veterans General Hospital, Taipei, Taiwan, ROC; [g]Department of Medical Research, China Medical University Hospital, Taichung, Taiwan, ROC; [h]Female Cancer Foundation, Taipei, Taiwan, ROC

Traumas and/or injuries are a nonavoidable events in all living things, including human beings. After a trauma or injury, some targets will die without any chance of compensation or regeneration and some of them will escape from death. These living things, if no immediate death occurs, have an immediate and quick initiation of a series of rescuing steps responding to these sub-lethal events, including a beginning of the compensation system to maintain the basic or alternative function of damaged organs and/or tissues and a subsequent driving of the repairing system (regenerative machines) to re-establish the physiological and morphological function of damaged tissues/organs finally. As a result, some are able to regenerate entire parts of the damaged tissues/organs, while some may be complicated by permanent damage even followed by those mild injuries.[1] The regenerative processes can be broadly classified into two categories: morphallatic and epimorphic.[1] The former involves migration of stem cells to the injury site, which differentiates into tissue-specific cells, and the latter involves the preexisting stem/progenitor cell or dedifferentiation of resident tissue cells,[1] and all are dependent on cell differentiation, dedifferentiation, and reprogramming processes. Regardless of either morphallatic- or epimorphic-type regeneration, the resident stem cell pools may be a crucial step toward a complete regeneration.[1] Unfortunately, human beings have very limited regenerative capacity, resulting in a loss of the potential for extensive organ repair and extremely restricted ability of self-regenerattion.[1] In the March issue of the *Journal of the Chinese Medical Association*, we are happy to learn Dr. Chiu's study exploring this hot topic—regeneration medicine.[2] The authors used an *in vivo* mouse model to evaluate the therapeutic effect of heparanase-1 on the bone healing process, and the results showed that topical application of heparanase-1 could accelerate the process of bone remodeling, with subsequently enhancing bone healing. The current study is impressive and worthy of discussion.

Address Correspondence. Dr. Peng-Hui Wang, Department of Obstetrics and Gynecology, Taipei Veterans General Hospital, 201, Section 2, Shi-Pai Road, Taipei 112, Taiwan, ROC. E-mail addresses: phwang@vghtpe.gov.tw; pongpongwang@gmail.com (P.-H. Wang).

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

Journal of Chinese Medical Association. (2020) 83: 695-696.

Received April 10, 2020; accepted April 10, 2020.

doi: 10.1097/JCMA.0000000000000330.

First, as shown by the authors, bone is the best tissue for the study of regenerative medicine, since minimal or scar-free repair of bone tissue can be achieved in the majority of cases of bone fractures.[2] The healing process of the bone follows the classical wound healing process, containing three separate stages, including hemostasis/inflammatory phase, proliferative phase, and remodeling, which is tightly controlled by orchestration of many factors to complete its healing process.[3–6] Although uncomplicated bone healing is frequently processed uneventfully, nonunion or poor union sometimes occurs, especially under the big defect of bone structure or infection status, and in addition, the immune-compromised host contributing to an enormous challenge concerning anatomic and functional integrity. After bone fracture, immediate placement or accumulation of blood clots and hematomas derived from the hemorrhage occurs, and it is an essential and critical step because this successful hemostasis acts as a scaffold for callus formation and as reservoirs trapping various types of inflammatory cells, immune cells, cytokines, and growth factors (inflammatory phase). The next step is the formation of granulation tissue by profuse osteoblasts, fibroblasts, macrophages, and an accumulation of extracellular matrix (ECM), such as proteoglycans, hyaluronic acid, collagen, elastin and others, to replace the original blood clots and hematomas, thus entering into the proliferative phase. All aforementioned phases are continuous, overlapped, and dynamic. Therefore, the following question is raised: Which component of the ECM is essential for the healing process? Is any component of the ECM good for the healing process? On obtaining the answer for the aforementioned questions, the next question is followed. When is the optimal time schedule to apply this essential to the injury sites? Is experiment-induced trauma or fracture similar to the nature- or accident-related trauma or fracture? The critical point is that the injury repair process is continuous and overlapped. Experiment-induced injury might involve many hand-made procedures. Hemostasis might be significantly different between experiment- and accident-related injuries. The blood clots and hematomas in the bone wound are apparently different between the two. All suggest that those questions are not easy to give an answer. However, this concept is very important for the further design of the study to investigate the biology.

In fact, the leading edge of the journal *Cell* published the full conversation between the 2020 *Canada Gairdner International Award* winner Mina Bissell and the Editor of the Journal Miao-Chih Tsai, which will give us more information. In fact, Dr. Mina Bissell established the brand new vision of biology, named as the "Dynamic-Reciprocity" view of biology, in which a bidirectional interaction between cells and their microenvironment is discussed, because her revolutionary works revealed the importance of ECM signaling and microenvironment on the gene expression, which explores the biological research beyond

genetic levels.[7] Dr. Mina Bissell explained that all living things do have all these trillions of DNA (deoxyribonucleic acid), and they are all the same; although, the nose and mouth are completely different, they have the same DNA.[7] So what on earth is telling the DNA what to do?[7] All suggest that ECM is really an overpowering regulator of the genetic information. Therefore, it is happy to learn that some researchers would like to focus on the investigation of ECM, like the article by Dr. Chang's group published in the March issue of the *Journal of the Chinese Medical Association*.[2]

Stem cell fate and expansion during repairing process, including branching morphogenesis, is regulated by the signaling provided by the local microenvironment (niche), which includes mesenchymal cells, nerves, blood vessels, lymphatics, and the growth factors bound to the ECM. Among the components of ECM, heparin sulfate (HS) is a negatively charged linear polysaccharide consisting of alternating glucosamine and uronic acid residues attached to a protein core of proteoglycans (HSPGs).[8] HSPGs, a large family of glycoproteins with a core protein, are highly variable in terms of number and size of HS moieties attached and can be further separated into at least three classes: (1) bound to the cell membrane by the glycosyl phosphatidyl inositol anchor (the glypicans); (2) contain a transmembrane domain (the syndecans and β-glycan); and (3) secreted into the basement membrane (perlecan, agrin, and collagen XVIII), and all are responsible to integrate autocrine and paracrine signals from the resident niche cells with signals for the HSPGs, together with signals from the stem cells themselves.[8] All suggest that complete and uneventful regeneration or morphogenesis need at least two components—cells and microenvironment.[9–12] Therefore, it is possible that HS chains of HSPGs are tissue-specific. In addition, HSPGs function as either a pro- or antiangiogenic factors, which can bind to and concentrate angiogenic growth factors in proximity to biologically active cell-surface receptors and, on the other hand, which can restrict the diffusion of growth factors within the microenvironment.[8] If the aforementioned hypothesis is possible, it is reasonable to explain why the application of HS to facilitate bone repair are not consistent as shown by the authors.[2]

Second, modification of HS can be made by either heparanase or sulfatase, which can function both intracellularly and extracellularly.[8] Similar to modification of sialic acid by sialyltransferases and sialidases,[13,14] this modification process of HS makes HSPGs reach an additional level of complexity to their role. The heparanase cleaves HS at many sites and is cooperated with matrix metalloproteinases to regulate ECM degradation and remodeling during the repairing process or branching morphogenesis.[8] However, it is interesting to find that knockout of heparanase does not result in any disturbance of repairing process or branching morphogenesis, although time course of healing process or branching morphogenesis is not investigated in detail. By contrast, is there any study to explore the relationship between overexpression of heparanase and wound healing process? One in vitro study showed that the overexpression of heparanase significantly reverses the inhibitory effect of unfractionated HA on osteoblast,[15] suggesting that continuous administration of heparanase might be beneficial in bone formation and fracture healing. It may support Dr. Chiu's finding—topical application of heparanase-1 facilitates bone remodeling during the healing of bone defect.[2]

Finally, in the Dr. Chiu's study, the drug delivery system should be introduced. An implantable osmotic pump (ALZET; Cupertino, CA, USA) can continuously deliver a fixed dose reagent topically through the catheter, which is widely used in the mouse model experiments with good results.[16] Since this powerful tool can provide a well-controlled drug delivery, the study to explore the effect of any new agent in the management of various diseases could be easily applied.

Healing is a relatively complicated process, and it involves cells and ECM. HSPGs actively contribute to cell-to-cell and cell-to-microenvironment signaling via multiple mechanisms and are influenced by the vast diversity of HS sequences, which are tightly controlled by heparanase and sulfatase. With the better understanding of their works, the successful healing process can be achieved. Good quality of healing responsible to various degrees of injury not only provides a restoration of anatomic structure but also offers a functional integrity. Since injury or ageing process cannot be avoided, more therapeutic implications for cell-mediated or mediators-mediated regeneration and morphogenesis are continuously welcomed.

ACKNOWLEDGMENTS

This article was supported by grants from the Ministry of Science and Technology, Executive Yuan, Taiwan (MOST 106-2314-B-075-061-MY3), and Taipei Veterans General Hospital, Taipei, Taiwan (V109C-108; V109E-005-5; V109A-022). The authors appreciate the financial support by Female Cancer Foundation, Taipei, Taiwan.

REFERENCES

1. Pesaresi M, Sebastian-Perez R, Cosma MP. Dedifferentiation, transdifferentiation and cell fusion: in vivo reprogramming strategies for regenerative medicine. *FEBS J* 2019;**286**:1074–93.
2. Chiu PY, HuangFu WC, Liu IH, Chang YP. Topical application of heparanase-1 facilitates bone remodeling during the healing of bone defects in a mouse model. *J Chin Med Assoc* 2020;**83**:272–9.
3. Chen GY, Chang CP, Wang PH. Burn wound and therapeutic challenge. *J Chin Med Assoc* 2019;**82**:748–9.
4. Wang PH, Huang BS, Horng HC, Yeh CC, Chen YJ. Wound healing. *J Chin Med Assoc* 2018;**81**:94–101.
5. Tsai HW, Wang PH, Tsui KH. Mesenchymal stem cell in wound healing and regeneration. *J Chin Med Assoc* 2018;**81**:223–4.
6. Horng HC, Chang WH, The CC, Huang BS, Chang CP, Chen YJ, et al. Estrogen effects on wound healing. *Int J Mol Sci* 2017;**18**:2325.
7. Asking the question of why. *Cell* 2020;181:503–6.
8. Patel VN, Pineda DL, Hoffman MP. The function of heparan sulfate during branching morphogenesis. *Matrix Biol* 2017;**57-58**:311–23.
9. Lee FK, Lin YL, Wang PH. Mesenchymal stem cells and cerebral palsy. *J Chin Med Assoc* 2020;**83**:323–4.
10. Li YT, Lee FK, Wang PH. Mesenchymal stem cells-derived magic bullets for burns. *J Chin Med Assoc* 2020;**83**:215–6.
11. Lin TC, Wang KH, Chuang KH, Kao AP, Kuo TC. Modulation of tumor stem cell characteristics by 17β-estradiol in human mesenchymal stem cells derived from ovarian endometrioma. *Taiwan J Obstet Gynecol* 2019;**58**:338–44.
12. Besikcioglu HE, Sarıbas GS, Ozogul C, Tiryaki M, Kilic S, Pınarlı FA, et al. Determination of the effects of bone marrow derived mesenchymal stem cells and ovarian stromal stem cells on follicular maturation in cyclophosphamide induced ovarian failure in rats. *Taiwan J Obstet Gynecol* 2019;**58**:53–9.
13. Lee WL, Wang PH. Aberrant sialylation in ovarian cancers. *J Chin Med Assoc* 2020;**83**:337–44.
14. Sung PL, Wen KC, Horng HC, Chang CM, Chen YJ, Lee WL, et al. The role of α2,3-linked sialylation on clear cell type epithelial ovarian cancer. *Taiwan J Obstet Gynecol* 2018;**57**:255–63.
15. Xia J, Sheng W, Pei L, Li N, Zhang Z, Wang J, et al. Effects of unfractionated heparin and rivaroxaban on the expression of heparanase and fibroblast growth factor 2 in human osteoblasts. *Mol Med Rep* 2017;**16**:361–6.
16. Wen KC, Sung PL, Hsieh SL, Chou YT, Lee OK, Wu CW, et al. α2,3-sialyltransferase type I regulates migration and peritoneal dissemination of ovarian cancer cells. *Oncotarget* 2017;**8**:29013–27.