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REGENERATION IN VERTEBRATES: A RESEARCH MODEL TO STUDY ANGIOGENESIS

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Abstract

Regeneration of animals' body parts has been observed for centuries. In vertebrates, research in regeneration has now produced a new level of understanding yet there are still many questions unanswered. One of the processes that commonly occur in regeneration is angiogenesis. Angiogenesis is the formation of new blood vessels from pre-existing blood vessels. Even though this process has been known to occur during regeneration but more research are needed to understand the whole process underlying angiogenesis during regeneration. One of the question which arise is what cause angiogenesis to occur only at a certain stage of regeneration and what process or signal activate the initiation of angiogenesis. This paper aims to review the importance of angiogenesis during regeneration and why the study of the regeneration process can be used as a model to study angiogenesis in vertebrates. Hopefully, more understanding on how this process works might help human to develop a method to cure or create new medicine to heal severe injuries both for human and othr vrtbrats as well.

Key words: *Vertebrates, Regeneration, Angiogenesis, Research Model*

INTRODUCTION

Regeneration is defined as a replacement of body parts (tissues, organ, appendages) that have been lost because of injuries (Bely and Nyberg, 2009; Poss, 2011). Regeneration is not confined to vertebrates only. Metazoan as well as vertebrates have this ability to repair and replace the injured parts o their body and even Plants also have regenerative ability through its "stem cells" (Birnbaum and Alvarado, 2008; Wullf, 2010). In vertebrates, regeneration is occurred throughout of all classes but the regeneration capacity of each class is quite different (Tsonis, 2000). Few groups of animal that have an outstanding regenerative ability are Fish, Urodeles, and Reptiles (Bely, 2010). Salamander is famous because of their ability to replace the lost limbs, eyes, jaw and even heart tissues (Tanaka, 2003). Lizards and Geckos are known to regenerate their lost tail (of autotomal process) into almost similar replica of the original tail. Fish also have similar ability to replace their lost appendages (fins), eyes and few organs. Those particular animal has been used as a model to study vertebrates regeneration for centuries (Nye et al., 2003). On the other hand, Mammals have been known to have an ability to replace certain parts such as Hepatic cells, Acinar cells or Pancreas or blood cells perfectly, but a complete limb or appendage replacement in adult mammals is never been observed (Tsonis, 2000).

Regeneration in Salamanders (Urodeles) consist of three stages which are : *Wound healing stage and Dedifferentiation*, *Blastemal Formation* and *Redifferentiation* which followed by *Organogenesis* (Iten and Bryant, 1976). Wound healing stage occurs right after injuries or autotomy happens. This stages were marked by the appearance of epidermal proliferation . Epidermal proliferation is the result of proliferating epidermal cells in order to close the wound. By the end o this stage, the wound will be closed entirely by layers of epidermal cells. Wound closing is important to prevent further infection and to protect the underlying cells which undergo a process called *Dedifferentiation*. During the dedifferentiation process, all cells that its fates has been determined previously will change into a group of cells that has no specific characteristic and act as embrionic cells . These cells are the cells that have an important roles during the next stages of regeneration and they are called the Blastema (Iten and Bryant, 1976, Tanaka, 2003).

The second stage of Urodeles and Reptiles Regeneration is called *Blastemal Formation*. This stage can only be found in epimorphic regeneration, type of regeneration occurs in urodeles, ish and reptiles (Endo *et al*., 2004). Blastema is a group of cells located beneath the epidermal cells. The origin of Blastemal cells has become a focus for research in regeneration since the formation of blastema will ensure the succes of regeneration. It has been shown that interuption of the dedifferentiation process and /or removing blastema from the regeneration site would delay the replacement of the lost body part hence prolong the regeneration time (Tanaka, 2003).

Blastema is formed by the dedifferentiation of tissues at the proximal part of the wound or injury. For years, it is believed that the injured parts (muscle, skin, bones and nerve) of the body contributed to the formation of blastema. Recent studies show that the most potent cells that contribute to the blastemal formation mostly comes from the muscle tissues (Tanaka, 2003). Why only muscle tissues having this multipotent abilities? What cause these tissues to dedifferentiate at the first place and what caused it to redifferentiate at the last stage of the regeneration ? Those questions are needed to be answered.

After the Blastema is formed, it will grow until it reach certain length. The length of Blastema depends on how much part of the body is lost during injury and/or autotomy. When the blastema has reach its maximum growth then the next stage will begin. The embrionic-like cells will then redifferentiated (redeveloped) into a spesific cells thus perform spesific function (Han *et al*., 2005). These cells then grow into tissues that had been lost during injuries. Blatema will form muscle tissues, bones, connective tissues and so on, until all of the parts that lost now replaced completely. This stage called the *Redifferentiation and Organogenesis*.

The unique and spesific sequence of events are the things that makes regeneration interesting to study. One event lead to another by particular signals and processes. One of the most important events that happened during regeneration is Angiogenesis. Why Angiogenesis is important and what is the function of Angiogenesis during Regeneration?

DISCUSSION

Angiogenesis during Regeneration

During regeneration , there is a particular process called *Angiogenesis*. Angiogenesis is the formation of new blood vessels from the pre-existing blood vessels. Research showed that Angiogenesis is activated since the beginning of the regeneration process, right after injuries or autotomy happened. Angiogenesis is needed to provide a way to transport all of the materials for the blastema to grow. Angiogenesis is differ from Vasculogenesis by means of its origin and process. While blastema still can be formed, without the new blood vessels , epithelial cells will not proliferate and the undifferentiated cells will not differentiate into cells with spesific functions (Seifert, et al. 2012). Yokoyama (2008) mentioned that the critical steps of

regeneration occurs at the beginning of it and during wound healing stages (the first stages o regeneration), most of the new blood vessels are formed through Angiogenesis.

In lizards tail regeneration, angiogenesis was initiated firstly during the beginning of wound healing stage. The rate of Angiogenesis will decline when blastema is formed, but the rate of Angiogenesis is going to be reincreased during the initiation of the third phase of regeneration. Molecular basis of cell-cell signaling and mechanism of angiogenesis activation are still being investigated. The disruption of angiogenesis (by radiation or injection of antiangiogenic factors) will delay the wound healing stage thus delay all regeneration stage (Putri and Rahman, 2008; Pillai, 2013).

Mammals can not perform a complete appendage regeneration, but Angiogenesis is a common physiological process that occurs in certain phase. In mammals, Angiogenesis normally occurs during wound healing, placental formation, menstrual cycle and embryo development, but the most studied event of Angiogenesis is in Tumor or Cancer development. During its development, Tumor will release certain factors that initiate the angiogenesis. The releasing of Tumor Angiogenic factor by Tumors will activate the initiation of angiogenesis. The new blood vessels are used by tumor to transport oxygen and food and all materials that needed by tumor to grow. So far, the knowledge of Angiogenesis has been used to produce medical treatment for people who suffer of certain type of tumor. For medicinal purpose, antiangiogenic factors for tumor has been developed as one alternative medicine to prevent tumor or cancer growth. The Question is : Is that medicine will target only the angiogenesis that happened around the tumor or it will affect the normal angiogenesis?

Angiogenic process in normal tissues such in regeneration has not yet become the focus of research particularly in regenerative medicine. Until now, researchers in the field of regeneration are far more interested on how to induce mammals tissues to have the same (or at least close to) regenerative ability as Salamanders and/or lizards. Those research mainly focused on how to trigger the response through experimentation on gene theraphy, stem and progenitor cell theraphy, reproggramming of the cell and tissue also tissue engineering yet the results are still unsatisfactory (Madeddu, 2005, Alvarado and Tsonis, 2006; Polykandriotis et al., 2010). The potential ability of mammals during the wound healing stage has been known for years, but the combination of this knowledge with the process of angiogenesis during regeneration may help us to create a method to fasten the healing of injury.

Why Study Angiogenesis in Vertebrates Regeneration?

As mentioned above, angiogenesis is important whether for regeneration or any other normal physiological events in the body. Studying angiogenesis will help us to understand certain physiological process of vertebrates body (including human), the development of certain disease and its possible use as a cure for particular diseases. Unfortunately, research in angiogenesis is limited to a few experiments in In Vitro study and it even harder to observe it In Vivo. Many methods of angiogenesis observation requires the sacrifice of the studied animals. For example, to study retinal angiogenic ability , studied animals has to be sacrificed only to harvest their eyes (Grant et al., 2002). In addition, In Vitro studies only give certain degree of observable results. Those results will be meaningless unless they were corroborated by In Vitro studies.

By using regeneration in amphibian (urodeles) and lizards the sacrife of animals can be minimized. It has been known that urodeles and lizards can regenerate their lost body part repeatedly. For example, lizards can perform autotomy as many time as possible. They also can grow back their tail as many time as they lost it (Seifert, et.al. 2012). The same things also happens in Urodeles. The other important thing regarding lizards ability is that, while there are visible changes in locomotor performance, losing their tail will not affect their ability to escape

predator (Maginnis, 2006; Bateman and Fleming, 2009; Higham et al., 2013). Some research show that certain species of lizards- that able to shed their tails- even become more elusive in evading their predator when they already lost their tail. Lizards and Urodeles also known to be able to shed their tail or appendages even if the previous regeneration has not been completed. Observation can be done only in regenerated parts. In order to harvest the part where angiogenesis occurs, researchers don't have to kill the animals. In my point of view (as a researcher), it means that the same animals can be used as many times as possible, thus minimized killing studied animals.

Ethical concerns and Animal Welfare

While urodeles and lizards might be beneficial to be used as animal model to study the process of angiogenesis during regeneration, their welfare as experimental subjects must be considered. The state of welfare of studied animals will determine the succes of observation and the error in experiment.In lizards and urodeles, even if the tail or appendages can be shed and replaced as many time as possible, there must be a limit for one animal to be used as subjects in experiments. Also, the bioethical requirements must be fulfilled : the state of the cage, cage environtment, food and drink and another substantial features to held animals in captive must be taken into consideration.

CONCLUSION

Angiogenesis is the common phenomena in vertebrates yet our understanding on this process is still limited. Regeneration can provide a way to researchers to study angiogenesis since during regeneration, Angiogenesis can be observed In Vitro without having to kill the animals. Vertebrate regeneration can be used as a model to study normal angiogenesis in animals while protecting and conserving the life and population of studied animals.

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