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Evidences of cell's pluripotency of hemichordata

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Abstract:

Hemichordata is one special kind of invertebrate animal that has many similarities with early vertebrate animals, especially the part of their dorsal column. Otherwise, hemichordata have a strong ability of regeneration. Regeneration is a sign which means the possibility of having totipotent adult stem cells. It is widely known that totipotent adult stem cells exist in many kinds of invertebrate animals, but they have not been found in the body of vertebrate animals in natural nowadays. Hemichordata may have possibility to be helpful due to its similarity of its dorsal column with early vertebrate animals. Dorsal column of vertebrate animals is one of the most complex structures in vertebrate animals' body, and hemichordata showed the possibility to regenerate in nature. Because of this, many attentions are caught by it. However, one important precondition of researches about totipotent adult stem cells of hemichordata has not been solved: the strong regeneration ability of hemichordata has not been proved completely, and controversy is still subsistent. This article will try to find some ways to prove the existence of hemichordata's totipotent adult stem cells in natural during its regenerative process.

Keywords: Hemichordata, totipotent adult stem cells, regeneration.

1. Introduction

People widely believe that animals that have totipotent adult stem cells are always invertebrate, such as Planaria and Hydra. However, vertebrate species, such as mammals, can only receive totipotent adult stem cells with the protection of labs [1]. Now adult stem cells with pluripotency from some kinds of mammals can be created with Yamanaka factors, which can be used to restore pluripotency in adult cells. Genes of Yamanaka factors are found in animals, which means that the ability to dedifferentiate and restore pluripotency in adult cells is possible in nature. Any finding of Yamanaka factors or their homologs that appear in nature always catches people's attention, especially when they appear in species that are close to mammals.

Therefore, the new discovery of hemichordata deserves attention. Through the examination of *Ptychodera flava* EST libraries, Pf-Pou3, Pf-SoxB1, Pf-Msxlx, and Pf-Klf1/2/4, which are found and believed in hemichordata like the acorn worm, are the only four known factors identified closely relating to Yamanaka factors [2]. Also, these factors are widely found at wounds of hemichordata [2]. It may be a breakthrough in research about factors that may affect the pluripotency of adult cells, similar to Yamanaka factors. Nevertheless, if cells of Hemichordata affected by these factors show pluripotency, it is not proven that these factors play a similar role to Yamanaka factors.

1.1 What is Hemichordata?

Hemichordata is a special kind of animal for the research of regeneration and pluripotency. With the research on hemichordata animals, people found many special features believed to only appear on chordate animals, such as pharyngeal gill slits, stomochords, and pygochords, which means that they may have a close affinity with chordate ones [3]. It is worth noting that they also show a strong ability for regeneration [3]. With the research of those factors similar to Yamanaka factors, the importance of hemichordata developed. The acorn worm is widely accepted as one kind of model organism to research for hemichordata, so it will be used as a model below.

However, compared with hemichordata's significance, the attention given to them is too low. There are still not many studies about hemichordatas, and so much information about them, for example, about how their cells were cultured artificially and whether they have any adult stem cells with pluripotency, still lacks consensus. The words below will give some evidence about whether hemichordata, like acorn worms, have adult stem cells with pluripotency due to factors.

1.2 Fate mapping

Fate mapping of cells is a method to establish the correspondence of cells between stages of development and later stages. Fate mapping is used as the bar code of embryo cells towards their own part of cells on the animal's adult [4]. With the marking of developing cells and tracing these marks, how these cells develop is clearly shown [5]. Fate mapping can be used to give evidence about whether stem cells have pluripotency, and it will be used in these two methods below. While marked cells appeared in different parts of the fate map, it can be any evidence to show the cell's pluripotency.

2. Methods

2.1 Method 1: Show the fate map of acorn worms (Figure 1).

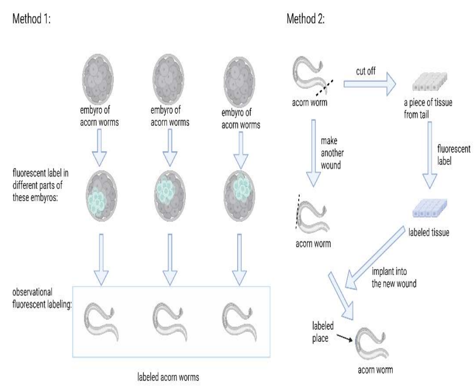

Figure 1. Embryo label in different parts

2.1.1 Method

In order to know the fate map of cells in acorn worms from embryonic stage to adult stage, cells at different parts of the embryos will be fluorescently labeled and traced in the worm's body.

For this method, three embryos are needed. Cells at different parts of these embryos are labeled with 5-bromo-2′-deoxyuridine and 5-ethynyl-2′-deoxyuridine (EDU) labels. This labeling can be used to visualize labeled cells (Life Technologies, New York, NY, United States). Then trace these cells during their growth and try to find out if the labeled cells can be found in different parts of the adult acorn worm's body.

2.1.2 Result

In this method, there is only one ideal result will happen below. If the appearance places of labeled cells gathered in some specific tissues of the acorn worm's body and labeled places of acorn worms who were labeled in different parts during embryo times are also found in different kinds of tissues, it means that the fate mapping can be found. Mark a part of the embryo and correspond to the labeled part of the adult worm. Matching different parts of embryos and adult worms one part by one part, and then the completely fate map can be found.

With the result of completing the fate mapping of acorn worms, more researches can be going on. If one kind of

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adult stem cell can develop into different kinds of tissue without the limitation of fate mapping, it can be proved have a higher possibility to have totipotence. In order to prove totipotence of an adult stem cell, fate mapping can give a large help on the choosing of cells and wound location to test in the next method.

2.2 Method 2: Show if the fate mapping of adult cells can be changed while they are in different parts of the worm's body (Figure 2).

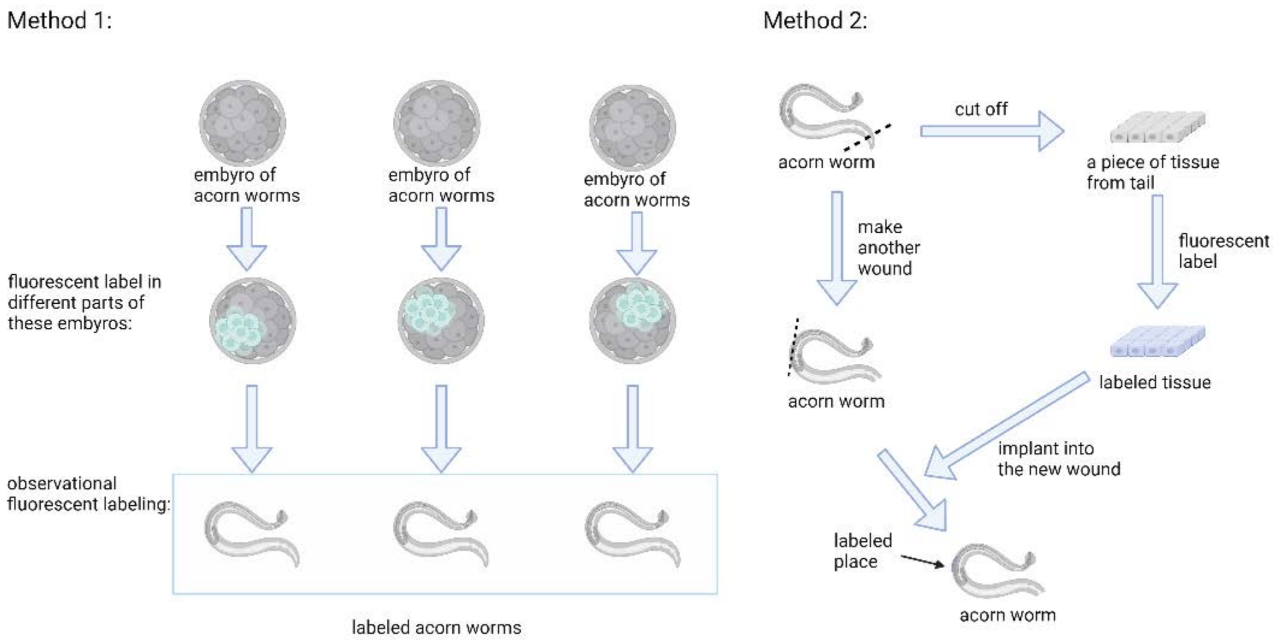

Figure 2. Labeling tissue in a wound from other part of body

2.2.1 Method

In order to know whether factors similar to the Yamanaka ones in this worm can also restore cell pluripotency, adult cells in one part of the worm's body will be moved to another part of the worm's body, and the moved cells will be traced.

For this method, one healthy adult acorn worm is needed. One small piece of tissue from its tail will be cut off and labeled with EDU labeling. Then, cut off another piece at the middle part of the worm's body and implant the marked tail tissue into the new wound. Trace the marked cells and observe the fate map of the labeled cells. If labeled cells can be found in the newly growing cells, it will be evidence that the roles Pf-Pou3, Pf-SoxB1, Pf-Msxlx, and Pf-Klf1/2/4 play on the pluripotency of acorn worm's cells are same as the roles Yamanaka factors play on the pluripotency of mammal's cells. Through all of these methods, cells should not be influenced by outside influences.

2.2.2 Result

In this method, several results may be watched. The ideal result shows that labeled tissue can develop as same as other cells of the new wound. In this situation, tissue cells can be differentiated as adult stem cells in nature. It can be easily found that the labeled tissue will join in the regeneration, and at last, many new cells of the new mound will be labeled. What is more, the shape of the worm's body is as same as the shape before the test. This result proves the tissue cells are not limited by fate mapping. These new adult stem cells have more possibility to be believed totipotent with this result.

Also, there are another two possible results. First, the labeled cell does not take part in regeneration. The number of labeled cells does not change, which means that tissue cells can not be regenerated in nature. However, rate of this result is quite smell. Also, labeled cells have the possibility to grow as a tail. This result means that adult stem cells can be created in nature, but these cells are limited by fate mapping. These cells are proved not have totipotence with this result. Both these two results will raise the possibility that stem cells in acorn worms' bodies do not have totipotent. If the result shows these two situations, the use of Pf-Pou3, Pf-SoxB1, Pf-Msxlx and Pf-Klf1/2/4 towards the body cells of acorn worms should be considered again.

3. Conclusion

Both of these two methods before should be used and they should be used in sequence. The first method which shows the fate mapping of acorn worms and give the next method a helping hand. In the second method, labeled tissue should not be chosen in the same fate mapping with the new mound, or it cannot be used to improve the totipotence of tissue cells.

However, the research about totipotent adult stem cells of hemichordata is also only the precondition of more research about hemichordata's regeneration. Hemichordata is still a kind of animal full of mystery with an important seat on evolution progress but less research. More research is necessary between the differences in stem cells between hemichordate and vertebrate animals, and more comparations are also necessary between Yamanaka factors and factors found in the body of hemichordate: Pf-Pou3, Pf-SoxB1, Pf-Msxlx, and Pf-Klf1/2/4. With this research, people will learn more about how evolution happened in the aspect of regeneration. People may use technology to regenerate as easily as totipotent animals within the learning of hemichordata in one day.

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