Müller glia-mediated retinal regeneration in irradiated embryos of zebrafish *(Danio rerio)*

(ゼブラフィッシュ胚における網膜の放射線傷害とミュラーグリアによる網膜組織の再生に関する研究)

動物生殖システム分野　沈蘊盛　47-206318

指導教員　尾田　正二

1. Introduction

The cells and structure of the retina are highly conserved in vertebrates (from fish to humans), and all vertebrates have retina with the three-layer structure (Stenkamp, 2007). It mainly consists of outer nuclear layer (ONL), which contains photoreceptors, inner nuclear layer (INL), which contains bipolar, horizontal, amacrine cells and Müller glia, and anglion cell layer (GCL), which contains ganglion cells. Embryonic retina of mammals, birds, reptiles and medaka are repaired after injury and the ability to repair the damaged retina will be lost during embryonic development and the adults have no or largely restricted ability. In contrast, even adult zebrafish can evoke the repair mechanism when retina is injured, making zebrafish an excellent model to understand retinal regeneration in vertebrates. The mechanisms of retinal regeneration in adult zebrafish have been extensively studied in decades, however, those in zebrafish embryo remains to be addressed due to the difficulty to reproducibly injure the small and rapidly developing retina in embryos. In this study, gamma-ray irradiation was employed to injure retina of zebrafish embryo and the process of retinal regeneration in zebrafish was investigated, especially focused on Müller glia behaviors, since Müller glia play the central roles in the retinal regeneration in adult and larval zebrafish (Bernardos *et al.*, 2007; Lust and Wittbrodt, 2018).

2. Results

2.1 Early development of retina in zebrafish embryos

In zebrafish embryos, the characteristic layered structure of retina began to develop at 49 hpf (hour post-fertilization) and ONL, INL and GCL were clearly recognized at 60 hpf. Müller glia first differentiated in the central part of the retina apart from the ciliary margin zone (CMZ) at 72 hpf.

2.2 Irradiation of zebrafish embryo with gamma-rays

29 and 50 hpf zebrafish embryos were irradiated with gamma-rays (10 Gy) and the effects on the retinal development was investigated. Many apoptotic cell deaths were induced in the retina of the zebrafish embryos at 22 hpi (hours post-irradiation) when the embryos were irradiated at 50 hpf.

2.3 Induction of apoptosis in irradiated retina of zebrafish embryo

Apoptosis was induced at 10 and 22 but not 48 hpi (Fig. 1 E-G), in contrast that no apoptosis was induced in non-irradiated embryos (Fig. 1 A-C). The apoptotic cell death were induced mainly in the INL and also in the ONL.

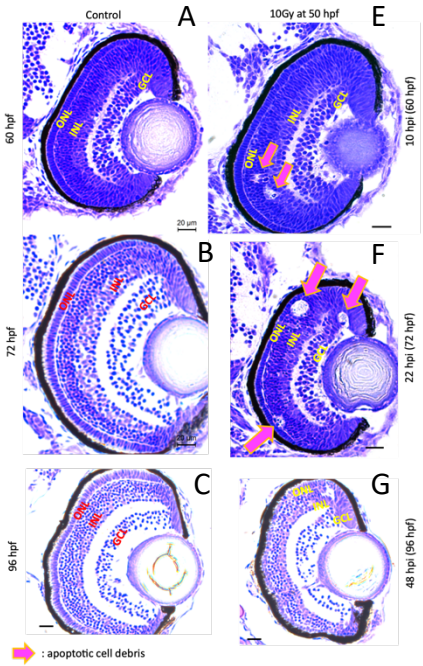

Figure 1　Apoptotic cell death (magenta arrows) induced in the retina of zebrafish embryos after irradiation at 10 (E) and 22 hpi (F) but not at 48 hpi (G).

2.4 Müller glia in INL ectopically proliferate in irradiated retina

In the retina of the non-irradiated embryos at 72 hpf, glutamate synthetase (GS)-positive Müller glia were aligned in the center part of the INL (white arrowheads in Fig. 2 A, C). In the retina of the irradiated embryos at 22 hpi, Müller glia left their normal position to migrate and surrounded apoptotic cells (white arrowheads in Fig. 2 A', C'). Proliferating cells were present only in CMZ in non-irradiated embryos (white unfilled arrows in Fig.2 B, C). In contrast, proliferating cells were present ectopically close to apoptotic cells in the INL and ONL in addition to in CMZ in the irradiated embryos. Müller glia in the irradiated retina changed their cell shape (yellow arrow in Fig.2 C') and some were proliferating both in the INL (yellow arrowhead in Fig. 2 C', yellow and magenta arrows in Fig. 2 D') and in the ONL (green arrows in Fig.2 D') after the irradiation.

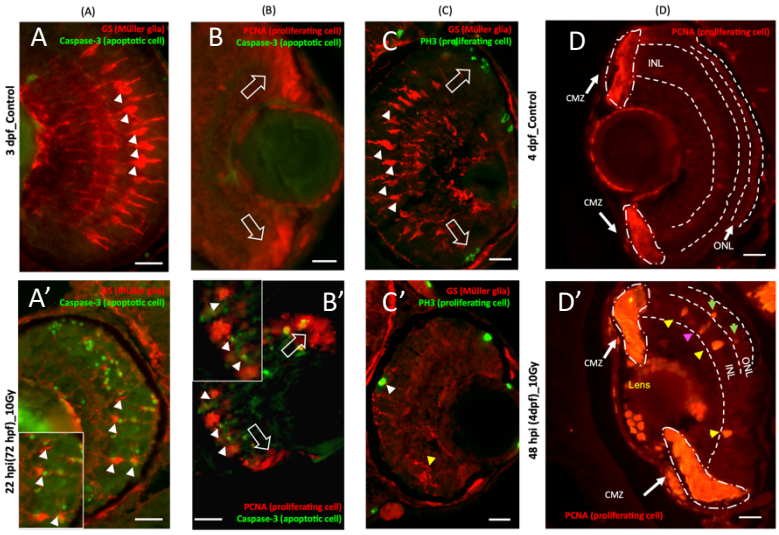

Figure. 2 Co-localization of Müller glia (red in A, A', C, C'), proliferating cells (red in B, B', D, D', green in C, C') and apoptotic cells (green in A, A' B, B') in non-irradiated (A, B, C, D) and irradiated (A', B', C', D') retina of zebrafish embryo at 22 hpi

2.5 Photoreceptor regeneration in irradiated zebrafish embryos

Alignment of the neural cells in the INL and ONL of the irradiated embryos were clearly disturbed 2 days after the irradiation. Some of the cells in the ONL with round cell shape might be progenitors for photoreceptor. On 3 days after the irradiation, the abnormal round cells in the ONL were still present (dark blue arrowheads in Fig. 3) and some of the adjacent cells showed the normal morphology of photoreceptors in a half of the irradiated embryos (red arrowheads in Fig. 3) and the ONL was restored to the normal structure, although the density of the photoreceptors in the ONL were looser than the non-irradiated embryos.

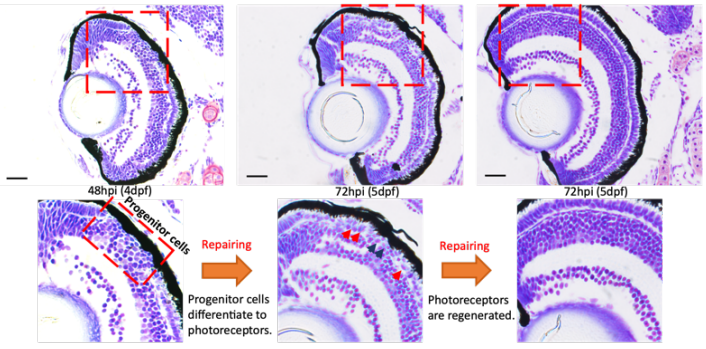

Figure.3 Photoreceptor regeneration process of zebrafish embryo after irradiation.

3. Conclusions

I employed gamma-ray irradiation to injure zebrafish embryos and established irradiation-induced retina injury model in zebrafish embryos. Gamma-ray irradiation induced apoptotic cell death both in INL and ONL and Müller glia played the central role in retinal regeneration in the injured retina after irradiation in zebrafish embryos. The retina regeneration process in zebrafish embryos might share the same process in the larval and adult zebrafish.