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Ultraviolet radiation and handling medium osmolarity affect chimaerism success in zebrafish

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Summary

The effects of a predefined ultraviolet radiation dose (0.529 mW/cm^2 for 30s) together with two different micromanipulation medium osmolarities (30 mOsm/kg vs 300 mOsm/kg) were tested on embryo survival at different developmental stages and on the somatic (skin) and germ-line chimaerism rates. Somatic (13%, 6/47 adults) and germ-line chimaerism (50% pigmented F1 larvae) were detected only in the UV-treated recipient embryos micromanipulated in a 300 mOsm/kg medium. From the results obtained, we concluded that the conditions cited above were the most suitable to improve somatic and germ-line chimaerism rates in zebrafish.

Keywords: Embryo, Germ-line chimaerism, Osmolarity, Zebrafish

Introduction

The chimaerism technique has proven useful to obtain offspring in which a part of the gametes comes from embryonic cells or embryo stem cells (Ma *et al*., 2001; Fan *et al*., 2004).

The colonization of transplanted cells in the presumptive chimaera, both at somatic and germ-line levels, depends on the preponderance of transplanted cells over the recipient cells. To facilitate colonization, different treatments can be applied with the aim of penalising the recipient embryo or some specific structures, as occurs with PGCs precursors (Carscience *et al*., 1993). To this end, chemical products (Swartz, 1980) or ionising radiations (Joly *et al*., 1999; Li *et al*., 2002) have commonly been used. The use of ultraviolet (UV) radiation is of interest because it is cheaper, easier and less dangerous than other kinds of radiation, and no special installations are required for its use. In our laboratory, a UV radiation dose to penalise recipient embryos was defined specifically for *wild* and *gold* zebrafish strains (Francisco-Simão *et al*., 2009). On the other hand, in another work, Cardona-Costa & Francisco-Simão *et al*., (2009) observed that the micromanipulation medium osmolarity (30 vs 300 mOsm/kg) could affect recipient embryo survival, possibly due to the rupture of the osmolarity barrier when the microinjection pipette punctured the outer embryonic layer.

In this context, the aim of the present work was to test the effect of the previously defined radiation dose (0.529 mW/cm^2 for 30 s) together with the micromanipulation medium osmolarity (300 or 30 mOsm/kg) on the germ-line chimaerism efficiency in zebrafish.

Materials and methods

Embryos at the early blastula stage from two different strains (*wild:* donors; *gold:* recipients) were used. All chemical products and culture media were from Sigma-Aldrich (Madrid, Spain)

UV irradiation of gold (recipient) embryos

According to previous results obtained in our laboratory (Francisco-Simão *et al*., 2009), gold-type embryos were treated with UV radiation to improve the

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colonization of transplanted cells during chimaeric embryo development. Briefly, embryo irradiation was carried out almost to mid blastula transition (MBT) stage without dechorionation. They were held in 35 mm-Petri dishes (corning) as containers with system water. A vortex (MS1-IKA) at 200 rpm was used with the aim of homogenising the radiation area during UV exposure. A UV germicide lamp (General Electric, 30 W) was used. Irradiation was carried out at 62 cm of focus-object distance. The radiation dose applied was 0.529 mW/cm^2 and was measured by a USB 4000 (Miniature Fiber Optic Spectrometer; Ocean Optics Inc. First in Photonics). After irradiation, embryos were kept at room temperature for 30 min and then dechorionated.

Chimaerism technique

Donor MBT blastomeres (non-radiated cells) from *wild* specimens were obtained by blastoderm disaggregation, in modified Hanks-buffered salt solution (HBSS) medium free of Ca^{2+} and Mg^{2+} (Cardona-Costa & García-Ximénez, 2007).

The chimaerism was performed using a Nikon inverted microscope (Nikon Europe B.V.) equipped with two Leitz micromanipulators (Leica). Two separated media drops were placed in a Petri-dish (90 mm) and covered by mineral oil. One of them was composed of HBSS (300 mOsm/kg) medium free of Ca^{2+} and Mg^{2+} contained the isolated blastomeres and the other one was the handling medium in which the chimaerism was performed, composed of HBSS (300 mOsm/kg) or HBSS-10% (30 mOsm/kg) medium (Pérez-Camps & García-Ximénez, 2008) depending on the experimental group carried out. During the manipulation process, the cells were picked with a microinjection pipette of 50 μm inner diameter and injected into the embryos held with a 260 μm outer diameter holding pipette. The number of injected cells per recipient embryo ranged from 50 to 100 cells and they were deposited into the animal pole as described by Lin *et al*. (1992); specifically, into the lower part of the blastoderm (Nakagawa & Ueno, 2003). Manipulated embryos were placed in 35 mm cell culture dishes at 28.5°C for 5 days in HBSS-10% (30 mOsm/kg).

Surviving embryos at 30–60 min were considered as the initial number. The further survival rates were assessed at 24 h, 48 h, 72 h and 5 days post-chimaerism. Then, surviving embryos were raised to adulthood where skin pigmentation from adult chimaera and their F1 progeny was registered.

Experimental design

Four experimental groups were established by combining embryo recipient UV radiation (30 s UV vs non-radiated) and the micromanipulation medium osmolarity (30 vs 300 mOsm/kg). Differences among groups in survival rates of different stages were tested. Somatic and germ-line chimaerism were evaluated in adults.

Overall germ-line chimaerism rate estimation

In this work, the parameter used to compare the osmolarity media and UV effect on germ-line chimaerism rates assumed that all the adult specimens obtained (male and female) in each experimental group provided a single 'hermaphrodite and simultaneous' gonad. In this way, depending on the treatment applied, the relative frequency of gametes produced (whether sperm or eggs) from donor cells (*wild*) or from recipient (*gold*) could be estimated by melanocyte presence in the larval skin, because the marker from *wild* specimens (pigmentation) is dominant over *gold* specimens, so only offspring from *gold–gold* gametes pairing will be non-pigmented. To this end, embryos from the four experimental groups were collected for 8 weeks and their skin pigmentation (*wild or* gold) was evaluated at 48 h developmental stage (Lin *et al*., 1992).

At least three replicates were done in all experimental groups. Results were analysed using the chi-squared test. When a single degree of freedom was involved, Yates correction for continuity was performed.

Results and Discussion

Results from Experiment I are shown in Table 1. In the irradiated groups, significant differences were observed in the survival rate at 24 h between the two handling media osmolarity (300 mOsm/kg: 50% and 30 mOsm/kg: 36%; $p < 0.05$). However, this difference gradually disappeared from the 48 h to 5 days stage, and even in the global survival (cumulative survival). Moreover, in the non-radiated groups, micromanipulation medium osmolarity did not affect embryo survival rates at any developmental stage. These results could indicate that the osmotic shock produced when chimaerism is performed in 30mOsm/kg micromanipulation medium osmolarity does not apparently affect long term survival in a relevant manner (Cardona-Costa & Francisco-Simão *et al*., 2009). As was expected, global survival (cumulative survival) rates in the irradiated groups were significantly lower than in non-irradiated (Francisco-Simão *et al*., 2009).

It may be noted that the number of males was higher than females in all experimental groups. No interpretation of this observation could be made because the system and factors of phenotypic sex

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Table 1 Survival rates of transplanted embryos. Embryos were irradiated or not (controls) and manipulated in different osmolarity media (30 and 300 mOsm/kg).

	30 s of UV		Non-radiated	
	300 mOsm/kg	30 mOsm/kg	300 mOsm/kg	30 mOsm/kg
Initial number of embryos	188	232	54	108
Normal embryos at 24 h (abnormal)	93/188 (50%)^{a} (30)	82/232 (36%)^{b} (38)	37/54 (69%)^{c} (6)	59/108 (55%)^{a,c} (16)
Normal embryos at 48 h (abnormal)	74/93 (80%)^{a} (21)	69/82 (84%)^{a,b} (31)	35/37 (95%)^{a,b} (4)	55/59 (93%)^{b} (9)
Normal embryos at 72 h (abnormal)	64/74 (87%) (22)	62/69 (90%) (24)	28/35 (80%) (3)	53/55 (96%) (5)
Normal embryos at 5 days (abnormal)	59/64 (92%) (12)	54/62 (87%) (23)	26/28 (93%) (5)	48/53 (91%) (3)
Global survival at 5 days	(31%)^{a} 59/188	(23%)^{a} 54/232	(48%)^{b} 26/54	(44%)^{b} 48/108

$^{a-c}$Columns with different superscripts are statistically different ($p < 0.05$).

Table 2 Sex distribution and pigmented marks in adult presumptive chimaeras.

	No. of final adult fish			No. of adults with pigmented marks		
Experimental groups	Total	Male	Female	Total	Male	Female
30 mOsm – non-UV	13	13	0	0	0	0
30 mOsm – 30s UV	11	7	4	0	0	0
300 mOsm – non-UV	9	7	2	0	0	0
300 mOsm – 30s UV	14	8	6	6	4	2
Total	47	74% 35/47	26% 12/47	13% 6/47	67% 4/6	33% 2/6

Table 3 *Wild* skin pigmentation rates in F1 larvae assessed at 48 h.

Experimental groups	Total embryos	Gold pigmented embryos	Wild pigmented embryos
30 mOsm – non-UV*	105	105	0 (0%)^{a}
30 mOsm – 30 s UV	25	24	1 (4%)^{a}
300 mOsm – non-UV	36	36	0 (0%)^{a}
300 mOsm – 30 s UV	494	254	240 (50%)^{b}

^{a,b}Data in rows with different superscripts are statistically different ($p < 0.05$).

*As all were males, gold females were introduced to make the germ-line chimaerism assessment feasible.

determination are unknown in zebrafish (Saito *et al.*, 2007).

Only six (four males and two females) from the 47 total adults showed *wild* skin pigmentation and all of them belonged to the 300 mOsm-30s UV experimental group (Table 2). Moreover, it should be emphasised that high rates of *wild* offspring (50%) were also only observed in the 300 mOsm–30 s UV group (Table 3). This fact confirms that the presence of pigmentation acts as an excellent sign of germ-line chimaerism in zebrafish (Lin *et al.*, 1992). In medaka fish, the gamma irradiation of recipient embryos also favoured the appearance of large pigmentation signals from donor cells and, in parallel, a significant increase in germ-line chimaerism (Joly *et al.*, 1999).

According to the results obtained, the penalization of recipient embryo with a radiation dose of 0.529 mW/cm^2 for 30 s together with the manipulation in 300 mOsm/kg handling medium osmolarity was the combination that obtained the best somatic and germ-line chimaerism rates.

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