World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(Research Article)

Check for updates

Loss of function phenotype in chicken embryonic development using the small interfering RNA method

Karamo Touré [1], Cé Camara [1,*], Ahmadou Sadio Diallo [1] and Yuan Li [2]

[1] *Department of Biology, Laboratory of Biochemistry, Faculty of Sciences, Gamal Abdel Nasser University of Conakry (UGANC), BP: 1147 Conakry, Republic of Guinea.*
[2] *School of Life Science, Laboratory of biochemistry, University of Xiamen, Fujian, People's Republic of China. PO Box: 361005.*

World Journal of Advanced Research and Reviews, 2022, 16(03), 648–654

Publication history: Received on 07 November 2022; revised on 19 December 2022; accepted on 21 December 2022

Article DOI: https://doi.org/10.30574/wjarr.2022.16.3.1383

Abstract

Introduction: The present study was based on the microinjection of lentiviruses into chicken embryos in the laboratory to demonstrate loss function phenotype in chicken embryonic development.

Method: This study aims primarily to demonstrate that small interfering RNAs can act effectively on chicken embryonic development by decreasing function of the chicken sphingosine 1 phosphate receptor 1/endothelial differentiation gene 1 (S1P1/EDG-1) during embryogenesis. In addition, chicken embryo fibroblasts (CEF) were prepared, plasmid gene 3132/PLL3.7 was used with 2XHBS for transfection, virus titer was determined prior to virus microinjection into chicken embryos to see the loss-of-function phenotype.

Results: The results obtained after viral microinjection showed that the function of S1P1/EDG-1 gene was progressively reduced from the second day of egg incubation until the fourth day. After the fifth day of incubation, loss of gene function was more marked by bleeding into the embryo sac.

Conclusion: Results from previous studies on other vertebrates such as mice have showed similar results [1, 2].

Keywords: Microinjection; Embryos; Phenotypes; Small interfering RNA; Transfection; S1p1/edg-1; Embryo fibroblasts

1. Introduction

Microinjection is one of the animal transgenesis techniques used in the laboratory to understand the expression of a gene such as small interfering RNAs in the embryonic development of certain vertebrates such as mammals, fish and birds. The animal model that was used in this study was the chicken. Lentivirus microinjection is a method of introducing genes of interest into a biologically relevant organism such as the chicken embryo. More recently, the chicken embryo has become particularly valuable in the study of gene expression, regulation and function. Microinjection of the virus in chickens can be used to show loss-of-function phenotypes with small interfering RNAs. For experimenters, chicken has a great advantage over mouse or rabbit because its embryo is accessible at all stages. Early blastoderms can be cultured in vitro long enough to form a recognizable primary body plan or sometimes manipulated in ovo and kept alive until later stages. Also, it is possible to explain small pieces of tissue on the chorioallantoid membrane of the later embryo, where they vascularize and will grow and differentiate in isolation. For these reasons, chicken embryo fibroblasts have been used to show loss-of-function phenotypes in chicken embryonic

* Corresponding author: Cé Camara

Copyright © 2022 Author(s) retain the copyright of this article. This article is published under the terms of the Creative Commons Attribution Liscense 4.0.

development [1, 2]. The aims of our study were to show that viral microinjection into chicken egg embryos using site 3132/Pll3.7 of the chicken S1P1/EDG-1 gene decreases the function of small interfering RNA in chicken embryo development as showed results of previous studies [1,2].

2. Materials and Methods

Cells: Chicken embryo fibroblasts (CEF) were prepared from chicken eggs on the fifth day after incubation. The head and limbs of the embryos were removed and then the embryos were washed in 1xPBS. CEFs were prepared by two cycles of trypsinization (1xtrypsin) for 2 minutes at 37°C. After centrifugation at 1100 rpm for 5 minutes, CEFs were cultured in DMEM with 10% NBS [3].

- Plasmid DNA: 3132, PLL3.7, PHR and VSV-G (vesicular stomatitis gene protein).
- Chicken eggs were kept at 37°C in the incubator.
- Reagents: 500 µl 2x HBS (physiological serum for herpes buffer), 50 µl of $CaCl_2$ 2.5 M, KCL: 0.38 g, NaCl…8g, Na_2HPO_4 …0.1g and glucose…1g. [3].

2.1. Methods

2.1.1. Preparation of plasmid DNA

Preparation of 3132 / pll3.7

Six (6) 10 cm boxes were used for 3132/pll3.7 so three (3) boxes for 3132 insert and three (3) boxes for the white pll3.7.

- 3132/pll3.7: 60µl
- PHR: 45µl
- VSV-G: 18µl
- 2.5M$CaCl_2$:150µl
- Distilled H_2O:1500µl

2.1.2. Preparation of 3132/pll3.7 plasmids

Twelve (12) boxes for 3132 and pll3.7 therefore six (6) boxes for the 3132 insert and six (6) for the Pll3.7 blank.

- PLL3.7/ 3132 20µg: 120µl
- PHR 15µg: 90µ
- VSV-G 6µg:36µl
- 2.5M $CaCl_2$ 50µl:300µl
- Distilled H_2O: 3000µl

2.1.3. Preparation of chicken embryo fibroblasts

- Put the chicken embryos in 2 ml, 1x PBS for washing.
- Add 2 ml of DMEM and centrifuge at 1100 rpm for 5 minutes.
- Remove the supernatant before adding 1.8 ml of DMEM and 2% FBS.
- Incubate the cell.
- After 24 hours, change the medium.
- Wash the cell with 1 ml 1 PBS.
- Add 1.8 ml of DMEM and 200µlFBS to the box before incubation at 37 degrees for injection of 5% CO_2. (See figure 1).

Six (6) plates for developing chicken embryo fibroblasts were prepared prior to virus conditioning. From liquid nitrogen, three (3) passes were made to obtain six (6) 10 cm dishes, three (3) for the 3132 packaging and three (3) for the pll3.7 packaging (See figure 1) [3].

2.1.4. Transfection process using 2xHBS

The 3132/PLL3.7 plasmids were prepared in six (6) dishes with 2xHBS (herpes buffered saline) to perform the transfection process.

- For 1500µl 3132 + 1500µl 2xHBS.
- For 1500µl pll3.7 + 1500µl 2xHBS.
- For 3000µl of 3132 + 1000µl 2xHBS.
- For 3000µl of pll3.7 + 1000µl 2xHBS.

All six (6) were incubated for 48 hours before using the fluorescence microscope for transfection results for insert (3132) and blank (pll3.7) respectively (see Figure 2)[3].

2.1.5. Lentivirus products

- Depending on transfection efficiency, virus supernatants were harvested two (2) times.
- First, virus supernatants were stored at 4°C overnight;
- Second, 10ml of DMEM was added to each dish before putting in the incubator for 12 hours. They were centrifuged at 75,000 rpm for 2 hours using a Beckman and Coulter centrifuge. After centrifugation, the supernatants were removed and the pellets were recovered with 200 µl of DMEM. We stored the tubes were stored at 4°C overnight. The next day we divided the virus obtained was divided into 2 ml tubes but each tube must contain 10 µl of virus or virus solution. The tubes of viral solutions were stored at -80°C [3].

2.1.6. Virus titer determination

Six (6) dilutions were used with six (6) well plates for chicken embryo fibroblasts for virus titer.

The different dilutions were 10-3, 10^{-5} and 10^{-7} thus three (3) well plates for each virus (3132/PLL3.7).

Well plates were incubated for 48 hours. For the viral insert 3132, the correct viral titer was obtained for a 10-3 dilution (see figure 3) and for the PLL3.7 blank, the correct viral titer was also obtained for a 10-3 dilution (see figure 4) (3).

2.1.7. Preparation of chicken eggs for microinjection

Thirty (30) chicken eggs were used for the viral microinjection, i.e. fifteen (15) eggs for the 3132 site and fifteen (15) eggs for the other pll3.7 site. The volume of egg white was reduced by approximately 2 ml so that it could expand to the butt end for viral injection. Eggs were incubated two (2) days prior to virus injection.

After the second day of egg incubation, microinjection of the lentivirus was performed. A volume of 10 µl of virus (3132/PLL3.7) was mixed with 1 µl of dye before microinjection. After the viral microinjection, the chicken eggs were incubated successively for two (2) days, for 72 hours, for four days and for five (5) days. This experiment was repeated two (2) times in a row before the confirmation of our results [3].

3. Results

3.1. Results for 3132 chicken S1P1/EDG1 gene sites

After viral microinjection into chicken embryos with the 3132 insertion site of the chicken s1p1/edg1 gene, the function of the gene decreased after the second day and third day and fourth day of incubation (see figures 5, 6, 7)but the bleeding into the embryo sac was more marked after the fifth day of egg incubation (see figure 8). Our results are comparable to other results from previous studies in mice and rats [4, 5, 6, 7, 8]. Virus microinjection using small interfering RNA (RNAi) to down-regulate the gene of interest in chick embryo development.

3.2. Results for Pll3.7 of the chicken S1P1/EDG1 gene

After the virus microinjection into chicken embryos with the PLL3.7 insertion site of the chicken S1P1/EDG1 gene, the function of the gene did not decrease during the embryogenesis process after the second day, third day, fourth day and on the fifth day of incubation (see Figures 9, 10, 11 and 12). This means that this lentivirus DNA has no insertion site in developing chicken embryo fibroblasts.

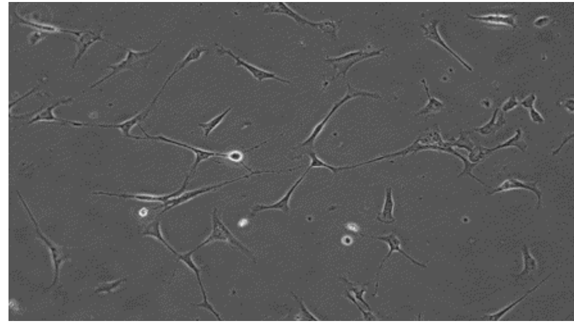

Figure 1 Chicken embryo fibroblasts

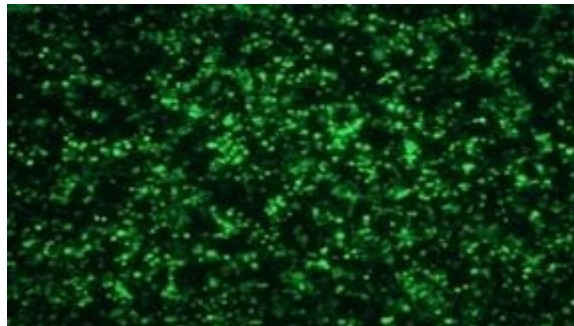

Figure 2 Efficiency of transfection results for site 3132/pll3.7 respectively after day 2 of incubation

Figure 3 Virus infection (3132) using developing chicken embryo fibroblasts (CEF) for a 10-3 dilution

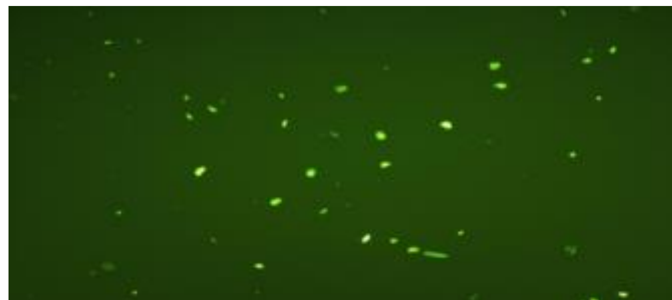

Figure 4 Virus infection (pll3.7) using developing chicken embryo fibroblasts at a 10-3 dilution

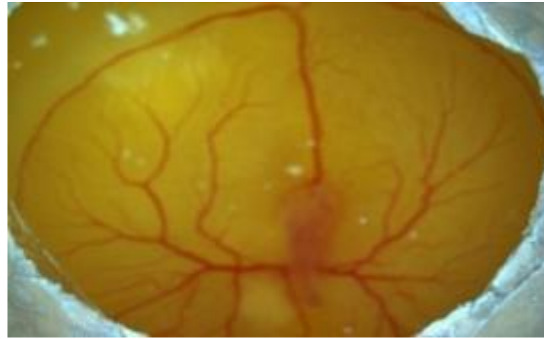

Figure 5 Loss-of-function phenotypes in chicken embryo fibroblasts after virus microinjection at day two for 3132

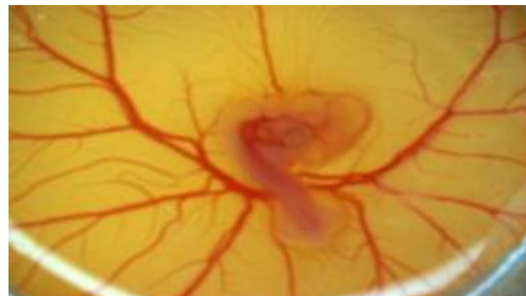

Figure 6 Loss-of-function phenotypes in chicken embryo fibroblasts after 72 hours of virus injection for 3132

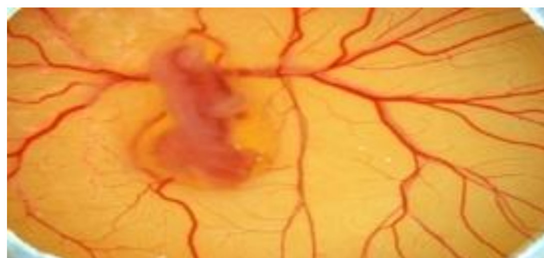

Figure 7 Loss-of-function phenotypes in chicken embryo fibroblasts after virus injection on day four for 3132.

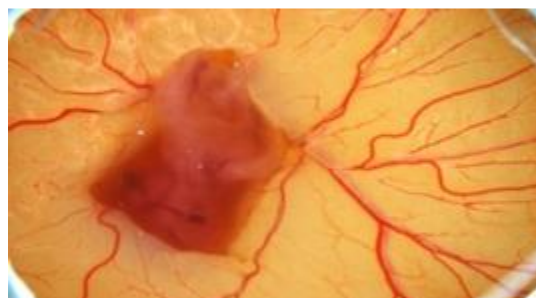

Figure 8 Bleeding into the yolk sac (downregulation) in chicken embryo after virus microinjection on day 5 for 3132

Figure 9 Normal embryogenesis in the developing chicken after virus injection on the second day for pll3.7

Figure 10 Normal embryogenesis in the developing chicken embryo after 72 hours of virus injection for pll3.7

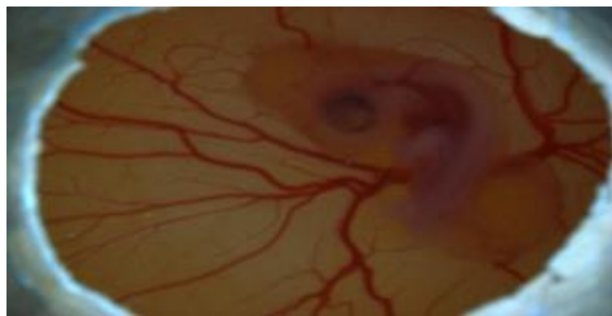

Figure 11 Normal embryogenesis in the developing chicken after viral DNA microinjection on day four for pll3.7

Figure 12 Normal embryogenesis in the developing chicken after viral DNA microinjection on day five for pll3.7

4. Discussions

The 3132/PLL3.7 site of the chicken S1P1/EDG1 gene produced lentiviruses capable of acting on chicken embryonic development with the immediate effect of loss-of-function phenotypes from the second day of egg incubation until the fifth day of incubation of chicken eggs after viral injection. This effect has been observed in other vertebrates, for example mammals [1, 2]. For the chicken S1P1/EDG1 gene insertion site 3132 the function of the gene decreased after the second day, third day and fourth day of egg incubation (see Fig. 5, 6, 7) but bleeding into the embryo sac was more pronounced after the fifth day of egg incubation (see Figure 8). For the PLL3.7 insertion site of the chicken S1P1/EDG1 gene, the function of the gene did not decrease during the embryogenesis process after the second day, third day, fourth day and fifth day of incubation (see fig. 9, 10, 11 and 12). Results from previous studies like ours have shown that micro

viral injection into chicken embryos is a small interfering RNA-based method to downregulation of the chicken S1P1/EDG1 gene [1, 2, 4, 5, 6.7, 8].

Abbreviations

- SiRNA: small interfering RNA
- CEF: chicken embryo fibroblasts

5. Conclusion

Viral microinjection into chicken egg embryos is the technique most used in the laboratory to understand the expression of a gene such as small interfering RNA. Virus microinjection into chicken egg embryos using the 3132/Pll3.7 site of the chicken S1P1/EDG1 gene decreases small interfering RNA function in chicken embryonic development as results from previous studies. For the 3132 insertion site, the loss-of-function phenotypes in embryonic development were not observed progressively from the second day of egg incubation until the fourth day. After the fifth day of incubation, the loss of gene function was more marked by bleeding into the embryo sac after viral microinjection followed by incubation of chicken eggs. For the PLL3.7 insertion site, loss-of-function phenotypes in embryonic development were not observed after viral microinjection followed by chicken egg incubation

Compliance with ethical standards

Acknowledgments

The authors are grateful for and acknowledge support from Department of Biology, Laboratory of Biochemistry of Gamal Abdel Nasser of Conakry (UGANC), BP: 1147- Republic of Guinea and the Laboratory of biochemistry, University of Xiamen, Fujian, Popular Republic of China. PO Box: 361005

Disclosure and conflicts of interest

The authors declare no conflict of interest.

Authors Contributions

All authors have contributed to this research and have read and approved the final version the manuscript.

References

[1] INRA animal productions 2000, R.Essalmani,S.Soulier, N. Besnard, M. Hudrisier, J. Costa Da Silva,J. L.Vilotte.

[2] INRA animal productions, 2017, Ducos A., Bed'hom B., Acloque H., Pain B.

[3] New cell lines for the production of lentivius. Lisbon September 2014. Diogo Lima from Vilhena Margalhas Costa.

[4] RNA interference towards functional genomics in mammals. 2004. Francois Dautry and Carole Ribet.

[5] RCAS-RNAi: A loss-of-function method for chick retina development © 2006 Harpavat and Cepko; licensee of BioMed Central Ltd.

[6] Zhang Y., Zhang Y.F., Bryant J., Charles A., Boado R.J. et Pardridge W.M . Intravenous RNA Interference Gene Therapy Targeting the Human Epidermal Growth Factor Receptor Prolongs Survival in Intracranial Brain Cancer. Clin Cancer Res. (2004) 10(11) :3667-77.

[7] Hasuwa H, Kaseda K, Einarsdottir T, Okabe M. Small interfering RNA and gene silencing in transgenic mice and rats. FEBS Lett. 2002 Dec 4;532(1-2):227-30. doi: 10.1016/s0014-5793(02)03680-3. PMID: 12459495.

[8] Transgenesis by lentiviral vectors: absence of gene silencing in mammalian embryonic stem cells and preimplantation embryos. December 18, 2001. Alexander Pfeifer, Masahito Ikawa, Yelena Dayn and Inder M. Verma*. Contributed by Inder M. Verma.