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(RESEARCH ARTICLE)

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Small RNA interfering effects in chicken embryonic fibroblasts using two sites 8664/p201 and 2122/pll3.7 of chicken s1p1/edg 1 gene

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Abstract

Introduction: Currently, short interfering RNA (siRNA) is an experimental tool in molecular biology to study gene function in cell culture and in vivo in model of organisms (mouse and chicken embryos, etc.)

Method: Lentivirus production technique by human embryonic kidney 293T cell lines (HEK293T) with two (2) sites 8664/P201 and 2122/Pll.3.7 of the sphingosine 1 phosphate receptor gene and endothelial differentiation 1 gene (S1P1/ Chicken EDG1) was used to show the effects of small interfering RNA in chicken embryonic fibroblasts during embryogenesis. After preparation of plasmids 8664/P201 and 2122/Pll3.7, lentivirus packaging, transfection with 2xHBS, virus titration and chicken egg preparation, microinjection of lentivirus was performed in the embryos chicken.

Results: The results obtained after the microinjection of lentivirus into chicken embryos from the second day to the fourth day of incubation showed the effects of small interfering RNA in the embryonic development of chicken materialized by bleeding in the yolk sac of embryos therefore a decrease in gene function.

Conclusion: Previous studies have already proven that expression of small interfering RNA decreases in embryos of certain vertebrates such as mammals.

Keywords: Lentivirus; HEK293T; Small interfering RNA; Embryonic fibroblasts; Micro injection; Virus titration

1. Introduction

Currently, short interfering RNAs (siRNA) are an experimental tool in molecular biology to study gene function in cell culture and in vivo in model organisms (mouse and chicken embryos, etc.) [1-3]. Double-stranded RNA (dsRNA) is synthesized with a sequence complementary to a gene of interest and introduced into a cell or organism, it is recognized as exogenous genetic material and thus activates a small interfering RNA pathway. Therefore, using the short interfering RNA mechanism, researchers has shown a drastic decrease in the function or expression of the targeted gene [1-4]. Studying the effects of this downregulation has shown a physiological role for this gene product, as small interfering RNAs may not totally abolish gene expression. Therefore, this method is sometimes referred to as "knock down" or downregulation, unlike "knock out" procedures in which expression of a gene is completely eliminated [1-9]. Knowing that the expression of small interfering RNAs has already been used during the development of the chick embryonic nervous system facilitated the performance of this study on chicken embryonic fibroblasts using microinjection of

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lentivirus [3]. The aims of this study were to demonstrate that the expression of small interfering RNA will decrease in chicken embryonic fibroblasts during embryogenesis after introduction of microinjection of lentivirus into embryos of chicken eggs from the second day to the fourth incubation day. For this recent study, two (2) areas 8664/P201 and 2122/Pll3.7 of the chicken S1P1/EDG1 gene were used to show the effects of small interfering RNA in chicken embryonic fibroblasts during embryogenesis after viral microinjection.

2. Material and methods

2.1. Material

Cells: HEK293T cell lines from the American Type Culture Collection (ATCC: Manassas, USA) under the designation "293", accession code CRL-1573 were provided [4].

Plasma DNA: PHR, VSV-G, 8664, P201, 2122 and PLL3.7 were purified using the PEG method (4). After purification of the plasmids, the concentration of each sample was 6 micrograms with a ratio of 1.8 [4].

Chicken eggs: stored at 37 °C before preparation for micro-injection of lentivirus.

Reagents: 2.5MCacl2, 500 microliter 2XHBS (herpes, 5g; NaCl, 8g; KCl, 0.38g; Na2HP04, 0.1g; glucose, 1g; and distilled water up to 500 microliters) and distilled water [4].

2.2. Methods

2.3. Plasmid preparation

- For 8664 and P201, six (6) boxes were used
  - P201, 8664 20µg 60µl
  - PHR 15µg 45µl
  - VSV-G 6µg 18µl
  - 2.5M CaCl2 50µl 150µl
  - Distilled water: 1500µl
- For 8664 and P201, twelve 12boxes were used
  - 8664/P201 20µg: 120µl
  - PHR 15µg: 90µl
  - VSV-G6µg: 36µl
  - 2.5CaCl2 50µl: 300µl
  - Distilled water: 3000µl
- For 2122 and pll3.7, six (6) boxes were used
  - The same concentration was used to insert (2122) and for the blank (Pll3.7)
  - 2122/PL3.7 20µg: 60µl
  - PHR 15µg: 45µl
  - VSV-G 6µg: 18µl
  - 2.5M CaCl2 50µl: 150µl
  - Distilled water: 1500µl
- For 2122/pll3.7, twelve (12) boxes were used
  - The same concentration was also used for the insertion of (2122) and for the blank (pll3.7
  - PLL3.7, 2122 20µg: 120µl
  - PHR15µg: 90µl
  - VSV-G6µg: 36µl
  - 2.5M CaCl2 50µl: 300µl
  - Distilled water: 3000µl

2.4. Lentivirus packaging

- HEK293T cell lines were placed in a 10 milliliter dish for packing when the cell density reached 70 or 80%. After obtaining 70% confluence, packing was done.

- Change the medium with 5 milliliters of pre-warmed DMEM (without serum) 2 hours before lentivirus packaging so as not to use 1xpbs to wash the cells;
- Prepare the plasmids in a 2 milliliter tube;
- Put the 2 milliliter tube on the vortex machine and add 500 microliter 2xHBS to the tube (plasmids) one drop at a time;
- After adding 500 microliters of 2xHBS (virus) put the lid back on the tube and mix before using the machine for one minute;
- Place the tube at room temperature for at least 5 minutes;
- Add to cell dish or plate one drop at a time and shake at the same time.
- Return the box to the incubator for 6-8 hours.
- Replace medium with 10-15 milliliters of DMEM (with 2% NBS) and incubate for 48 hours (calculated from reagent addition to dish).
- Take up the medium and filter it through a 0.45 micrometer filter into a 50 milliliter tube.
- Spin at 75,000 rpm for 120 minutes or 2 hours.
- Remove the supernatant and add 100-200 microliters of DMEM to the tube before placing it at 4°C overnight.
- The next day, separate the liquid into 0.2 milliliter tubes with 10 microliters each [4].

2.5. Transfection with 2xHBS

- Six (6) boxes for 8664 and p201 were used:
  - For insertion: 1500µl 8664 + 1500µl 2xHBS.
  - For white: 1500 µl P201 + 1500 µl 2xHBS.
- Twelve (12) boxes for 8664 and p201 were used:
  - For insertion: 3000 µL 8664 + 3000 µl 2xHBS (see figures 1 and 2).
  - For white: 3000 µl P201 + 3000 µl 2xHBS [4].

2.5.1. Virus titration

Virus titer for 8664 and P201

Six (6) dilutions were carried out, three for the insertion and three for the blank to know the titer of the virus therefore the dilutions were respectively 10^{-3}, 10^{-5} and 10^{-7}.

For the first well plate, 999 µl of DMEM, 10% NBS, 1 milliliter of HEK293T cell lines were used.

1 µl of virus was used for the first well plate after 10 µl of the first well plate for the second well plate, 10 µl of virus from the second well plate for the third well plate and so on [4].

After the second day of incubation, the correct titer was obtained with a dilution of 10^{-5} (see figure 3) [4].

2.5.2. Preparation of eggs for virus injection

For 8664 and p201

First, 2 ml of egg white was removed before gluing the pit with dye so that the chicken embryos could develop at the butt, which made it easy to obtain the embryos during microinjection of lentivirus.

Second, eggs were incubated for 48 hours before microinjection;

Third, after incubation, microinjection of the virus into chicken fibroblasts was performed to see the expression of small interfering RNA.

2.5.3. Injection of the virus into chicken embryonic fibroblasts

For 8664 and p201

Ten (10) µL of virus was used for virus microinjection but 1 µL of dye was added for staining.

The viral micro-injection was done after two (2) days of egg incubation.

Fifteen (15) eggs were used for 8664 and for p201 each and this experiment was repeated three (3) times to confirm the results.

2.6. Transfection with 2xHBS

- Six (6) boxes were used for 2122 and PLL3.7
  - 1500µl 2122 + 1500µl 2xHBS.
  - 1500 µl of PLL3.7 + 1500 µl of 2xHBS.
  - Twelve (12) boxes were used for 2122 and PLL3.7
  - 3000 µl 2122 + 3000 µl 2xHBS for insertion (see figures 8 and 9).
  - 3000 µl PLL3.7 + 3000 µl 2xHBS for white [4].

2.6.1. Virus titration

Virus titer for 2122 and pll3.7

Six (6) dilutions were used, three (3) for the insertion and three (3) for the blank to know the title of the virus therefore the results obtained were respectively 10^{-3}, 10^{-5} and 10^{-7} .

For the first well plate, 999 µl of DMEM, 10% NBS, 1 milliliter of 293T cells were used.

1 µl of virus for the first well plate, 10 µl of virus from the first for the second well plate and 10 µl of virus from the second plate for the third well plate, and so on.

After the second day of incubation, the correct titer was obtained with a dilution of 10^{-5} (see figure 10) [4].

2.6.2. Preparation of eggs for virus injection

For 2122 and pll3.7

First, 2 ml of egg white was removed before gluing the pit with dye so that the chicken embryos could develop at the butt, which made it easy to obtain the embryos during microinjection of lentivirus.

Second, eggs were incubated for 48 hours before microinjection;

Third, after incubation, viral microinjection into chicken fibroblasts was performed to see the expression of small interfering RNA.

2.6.3. Injection of virus into chicken embryonic fibroblasts

For 2122 and PLL3.7

Ten (10) µl of virus was used for virus microinjection and 1 µl of dye was added for staining.

The lentivirus microinjection was made after 48 hours of egg incubation.

Ten (10) eggs were used for 2122 and for pll3.7 and this experiment was done three (3) times to confirm the results [4].

3. Results

3.1. Results for 8664 and P201

After viral microinjection into chicken egg embryo fibroblasts, for the 8664 site, it was observed the loss of phenotypic function or gene function decreased in chicken embryo fibroblasts with the 8665 insertion gene at the course of embryogenesis from the second day to the fourth day (see Figures 4 and 5).

For P201, the blank, there was no effect so RNAi function was not impaired (see Figures 6 and 7).

For this study, the effects of small interfering RNA were demonstrated in chicken embryo fibroblasts obtained using HEK293T cell lines using two (2) sites (8664 and 2122) of the chicken S1P1/EDG1 gene.

3.2. Results for 2122 and Pll3.7

After viral microinjection into chicken embryo fibroblasts, for insertion site 2122, it was observed the loss of phenotypic function or decreased gene function in chicken embryo fibroblasts during embryogenesis of the second day to fourth day (see Figures 11 and 12).

For the P113.7 site, the blank, there was no effect because small interfering RNA function was not decreased (see Figures 13 and 14).

For this study, the effects of small interfering RNA were shown in chick embryo fibroblasts obtained using HEK293T cell lines using two (2) sites 8664/P201 and 2122PL3.7 of the chicken S1P1/EDG1 gene.

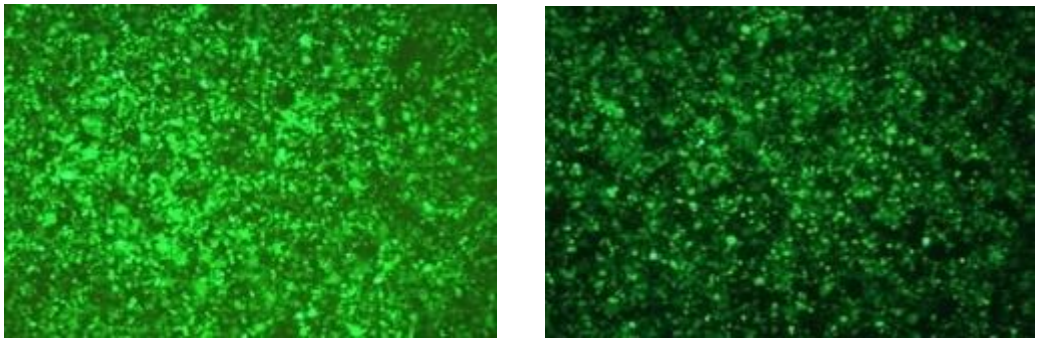

Figure 1 Transfection efficiency for 8664, 10x and p201, 10x after 48 hours of incubation for six (6) plates prepared respectively

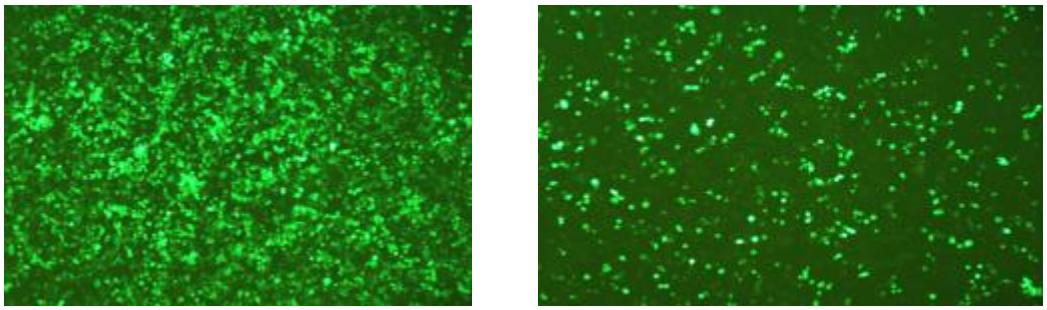

Figure 2 Transfection efficiency for 8664, 10x and p201, 10x after the second day of incubation with twelve (12) plates prepared respectively

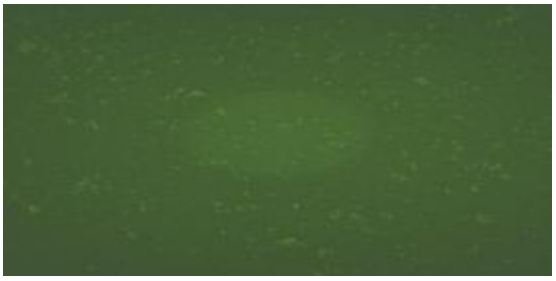

Figure 3 Viral titer of site 8664 for the 10^{-5} dilution

Figure 4 Chicken embryonic fibroblasts showing bleeding in the yolk sac after injection of virus 8664 on the second day of incubation

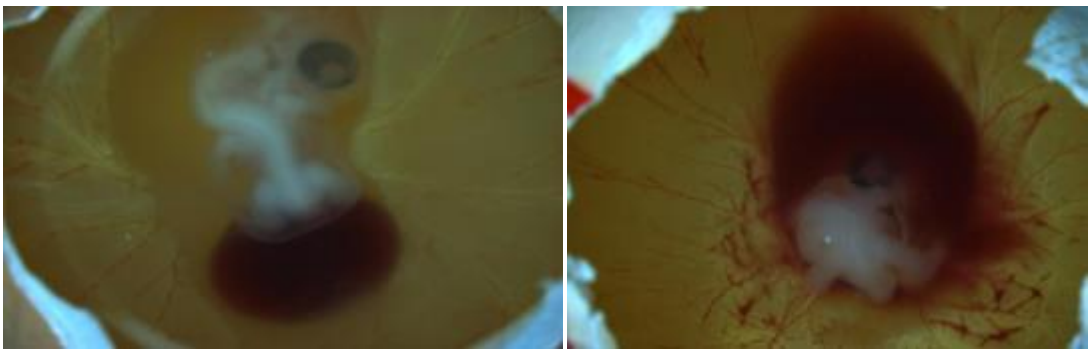

Figure 5 Chicken embryo fibroblasts showing yolk sac bleeding (downregulation) after virus 8664 injection on day 4

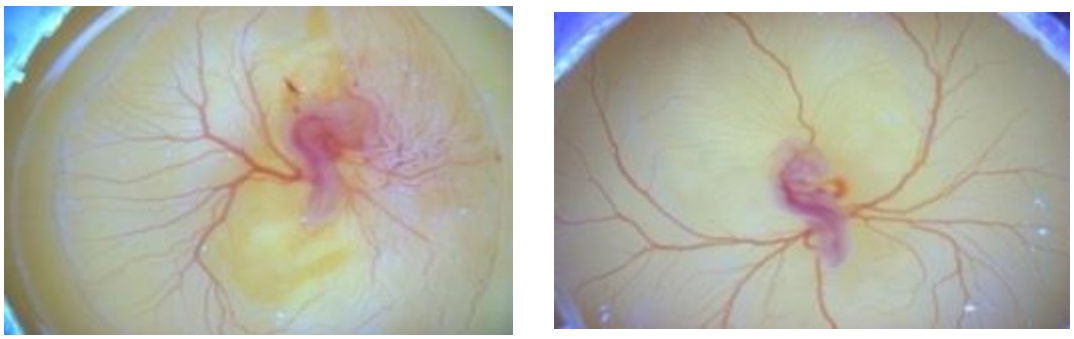

Figure 6 Chicken embryo fibroblasts showing normal embryogenesis after virus injection on day 2 for the P201 site

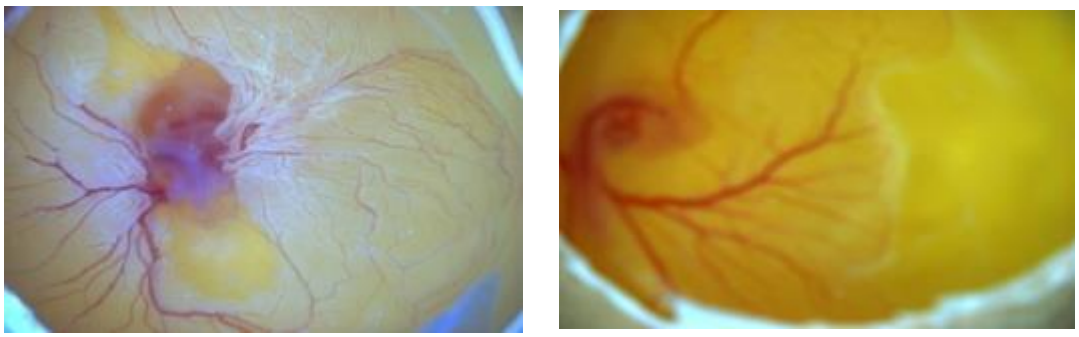

Figure 7 Chicken embryo fibroblasts showing normal embryogenesis after virus injection at day 4 (P201)

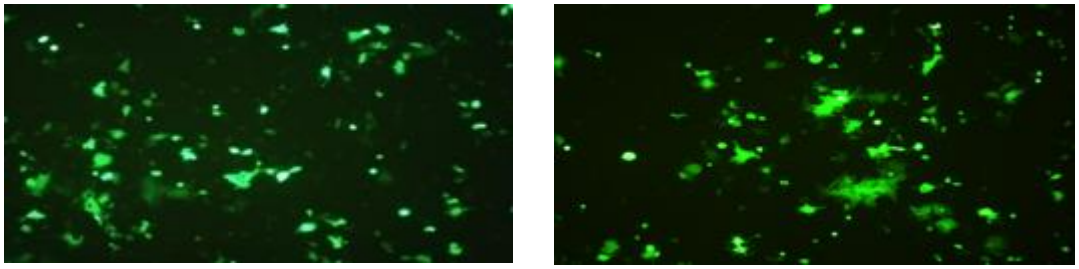

Figure 8 Transfection efficiency after day 2 for 2122, 10x and p113, 7, 10x for six(6) dishes respectively

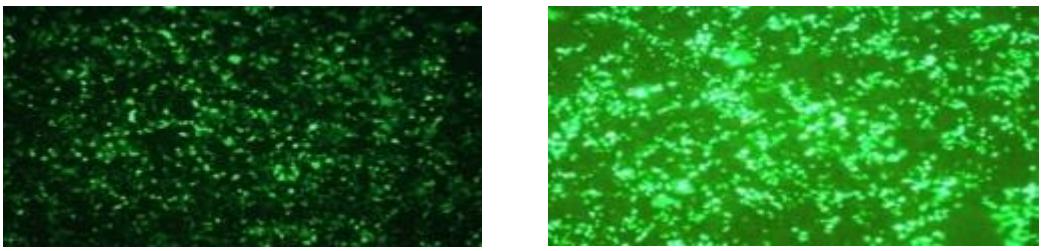

Figure 9 Transfection efficiency for 2122, 10x and p113.7, 10x after day 2 incubation for twelve (12) dishes respectively

Figure 10 Viral titer of site 2122 for the 10^{-5} dilution

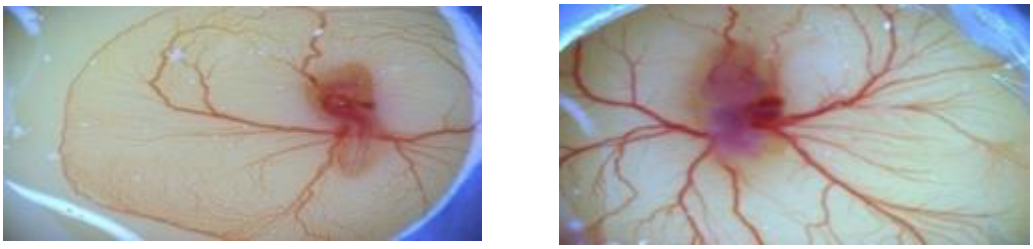

Figure 11 Chicken embryos showed downregulation of the gene after injection of virus 2122 on day 2

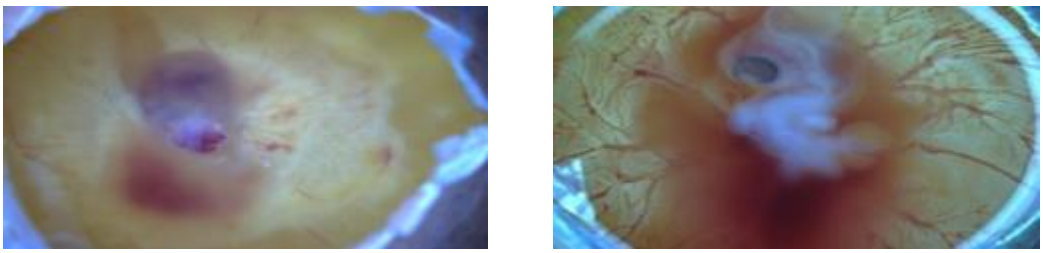

Figure 12 Chicken embryo fibroblasts showed bleeding into the yolk sac after day 4 of virus injection (2122) respectively

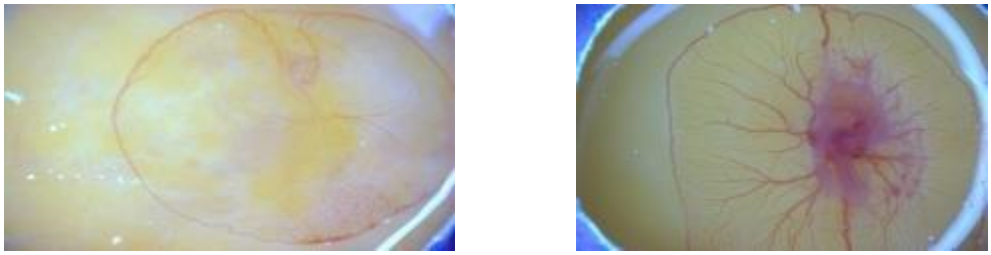

Figure 13 Chicken embryo fibroblasts showed normal embryogenesis after virus injection at day 2 for the PLL3.7 site

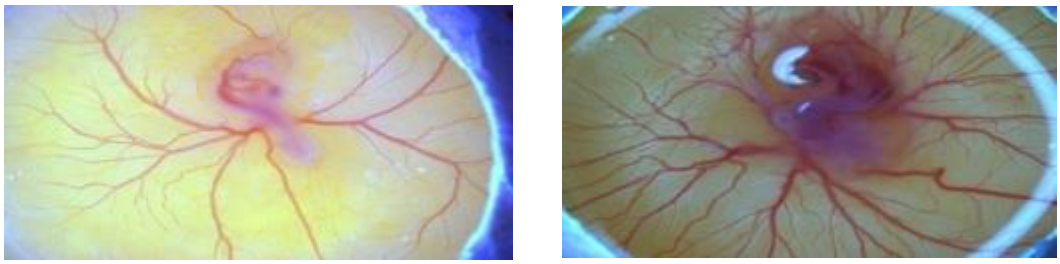

Figure 14 Chicken embryo fibroblasts showed also a normal embryogenesis after virus injection at day 4 for the PLL3.7 site

4. Discussions

The decrease in small interfering RNA expression observed during embryogenesis in chicken embryonic fibroblasts is comparable to other previous findings observed in the development of the chicken embryonic nervous system with the loss-of-function method for the development of the chicken retina unto the expression of micro RNA during the development of chicken embryos [2, 3, 5].

The effects of small interfering RNA in chicken embryos fibroblasts have been observed during embryogenesis by bleeding into the embryo sac, a similar pattern were fund into small interfering RNA action and gene sequencing in mice and transgenic rats [5].

The two (2) sites 8664/P201 and 2122/PLL3.7 of the S1P1/EDG1 gene produced lentiviruses capable of acting on the embryonic development of chicken with immediate effect of reducing the function of small interfering RNA from the second day unto the fourth day of incubation of chicken's eggs after viral injection. This effect has been observed in other vertebrates [2, 3, 5, 6,7].

Alongside, previous studies showed similar results to ours; the small interfering RNA is a better method that mediates gene expression in vertebrates [1-9].

5. Conclusion

Lentivirus production by HEK 293T cell lines with two (2) sites 8664/P201 and 2122/PLL3.7 of the chicken S1P1/EDG1 gene followed by microinjection showed decrease in small interfering RNA expression (RNAi) in chicken embryonic fibroblasts during embryogenesis. HEK293T cell lines were used to produce lentiviruses using two chickens S1P1/EDG1 gene plasmids 8664/P201 and 2122/PLL3.7 after conditioning. Transfection efficiency and normal viral titer using different dilutions were obtained by first conditioning with the site 8664/P201 of chicken S1P1/EDG1 gene. Viral microinjection into chicken egg embryos resulted in a decrease in small interfering RNA function in embryonic fibroblasts during embryogenesis. Transfection efficiency and normal viral titer using different dilutions were obtained by second conditioning with the 2122/PLL3.7 site of the chicken S1P1/EDG1 gene. Viral microinjection into chicken egg embryos resulted in a decrease in small interfering RNA function in embryonic fibroblasts during embryogenesis. The fluorescence microscope was used to visualize the transfection efficiency for the two (2) experiments and the pictures of all chick egg embryos were taken using the same machine.

The magnification was 0.8 for each embryo observed. The creation of study conditions to facilitate the performance of such work in our country, the Republic of Guinea, will be an opportunity in the future to deal with viral infections in chickens.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict 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] RNA interference towards functional genomics in mammals. 2004. Francois Dautry and Carole Ribet.

[2] RCAS-RNAi: A loss-of-function method for chick retina development. 2006, Harparat and Cepkolicenseebiomed central Ltd.

[3] Downregulate the genes of interest during the development of the embryonic nervous system of the chicken. (2003Pekarik et al) and(2003Stoecki).

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

[5] Small interfering RNAs and gene silencing in transgenic mice and rats. 2002. Hidetoshi Hasuwaa, Kazuhiro Kasedab, Thorbjorg Einarsdottira, Masaru Okabea;

[6] 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.

[7] SiRNAlentiviralsilencing of S1P1 and S1P2 sphingosine-1-phosphate receptors in smooth muscle. 2006 Biochemical and Biophysical Research Papers 343 (1038–1044.

[8] Design of non-inflammatory synthetic siRNA mediating potent gene silencing in vivo. *. Available online December 15, 2005. Adam D. Judge, Gurneet Bola, Amy C.H. Lee, Ian Mac Lachlan.

[9] Expression of microRNAs during the development of chick embryos. Dynamic Development 235: 3156–3165, 2006.