mRNA Expression of the Stem Cell Markers in the Normal Rat Lens

Konstantin Galichanin*

Department of Surgical Sciences, Gullstrand Lab, Section of Ophthalmology, Uppsala University, Uppsala, Sweden

Corresponding Author*

Konstantin Galichanin
Department of Surgical Sciences,
Gullstrand Lab, Section of Ophthalmology,
Uppsala University, Uppsala, Sweden
Tel: +46186113716
E-mail: konstantin.galichanin@neuro.uu.se

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Abstract

Purpose: This study aims to investigate the expression levels of stem cell markers in the normal lens of rats.

Methods: Ten six-week-old female albino Sprague-Dawley rats were used in this study. After euthanasia, their lenses were extracted and divided into two components: Lens epithelial cells and lens cortical fiber cells. The presence of stem cell markers *nestin*, *NGFR*, and *ABCG2* in both regions was analyzed using reverse transcription quantitative polymerase chain reaction (RT-qPCR). The relative mRNA expression levels of these markers were compared between the two lens regions.

Results: The analysis revealed that *nestin* expression in lens epithelial cells was 229 times higher than in the lens cortical fiber cells. Similarly, *ABCG2* showed a 23 fold increase in the epithelial cells. In contrast, *NGFR* was slightly more expressed in the epithelial cells, with a 0.9 fold difference compared to cortical fiber cells.

Conclusion: The findings indicate that *nestin*, *NGFR* and *ABCG2* exhibit higher expression levels in rat lens epithelial cells than in cortical fiber cells.

Keywords: Rat lens • *Nestin* • *NGFR* • *ABCG2* • RT-qPCR

Questions

What is already known?

Previous research has examined the expression of stem cell markers in the mouse lens. This study shifts the focus to the normal rat lens.

What does this study contribute?

It identifies a significant upregulation of *nestin*, *NGFR* and *ABCG2* in lens epithelial cells compared to cortical fiber cells.

What are the potential implications?

The findings may aid in advancing our understanding of the biology and development of the rat lens and could have broader implications for regenerative medicine or lens-related pathologies.

Introduction

This study aims to examine the expression patterns of specific stem cell markers within the normal lens of rats.

A search of existing literature in PubMed reveals a lack of studies focusing on the presence of these markers in the rat lens, although such markers have been explored in the context of the mouse lens [1]. It is known that stem cells are located in the germinative zone of the lens [2], which provided the rationale for undertaking this investigation.

Historically, stem cells were first identified in bone marrow. In recognition of pioneering work in this field, E. Donnall Thomas and Joseph E. Murray received the Nobel Prize in 1990 in recognition of his significant work on organ and cell transplantation used in the treatment of human illnesses. Stem cell research continues to be a cornerstone of biomedical studies around the world. In ophthalmology, stem cells have been employed in research related to cataract repair and lens regeneration in both animal models and human subjects [3].

Nestin is an intermediate filament protein typically found in proliferating cells during early development. It is expressed in the central and peripheral nervous systems, muscle tissue and other developing organs. This protein plays a crucial role in maintaining self-renewal, guiding cell differentiation and supporting cellular migration by interacting with the cytoskeleton [4].

NGFR (Nerve Growth Factor Receptor) acts as a receptor for neurotrophic factors and is considered a stem cell marker in various tissues such as esophageal keratinocytes, retinal cells and adipose-derived cells [5-7]. It is implicated in processes like neuronal differentiation, survival, programmed cell death and neurite extension.

ABCG2 belongs to the ATP-Binding Cassette (ABC) transporter protein family. It contributes to cellular defense against xenobiotics and is an essential component of physiological barriers such as the blood-brain and maternal-fetal barriers. Its presence in stem cells suggests a protective role in maintaining stemness and cellular integrity [8].

Given this background, the current study was undertaken to explore the expression of *nestin*, *NGFR*, and *ABCG2* in the normal rat lens, contributing to a deeper understanding of lens biology and potential regenerative mechanisms.

Materials and Methods

Animals

The study was conducted using six-week-old female albino Sprague-Dawley (SD) rats, sourced from Taconic, Denmark. All procedures involving animals adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research. Ethical approval for the experiment was granted by the Uppsala Animal Ethics Committee, under protocol number C29/10.

RT-QPCR

Total RNA was extracted using the NucleoSpin RNA II kit (Macherey-Nagel GmbH and Co., Düren, Germany). RNA concentration and purity were determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Products, Wilmington, DE, USA), based on the 260/280 absorbance ratio. Complementary DNA (cDNA) was synthesized from 1 µg of total RNA using the 1st strand cDNA synthesis kit (Roche Diagnostics GmbH, Mannheim, Germany).

Quantitative Real-Time PCR (RT-qPCR) was carried out using an iCycler MyiQ Single Color Real-Time PCR detection system (Bio-Rad Laboratories, Hercules, CA, USA). TaqMan gene expression master mix (Applied Biosystems, Foster City, CA, USA) was used in combination with specific TaqMan assays for *nestin* (Rn00564394_m1), *ngfr* (Rn00561634_m1), *abcg2* (Rn00710585_m1) and 18s (Hs99999901_s1), following the manufacturer's protocol. The MyiQ software algorithm (Bio-Rad) analyzed primary fluorescence and a standardized threshold was applied to each PCR plate to determine the Cycle Threshold (CT) values.

Experimental design

Ten rats were utilized for the experiment. After euthanasia, the lenses were removed and separated into two specific regions: The lens epithelial cells and the cortical fiber cells. RNA was then extracted from both regions and subjected to RT-qPCR analysis to assess the expression levels of *nestin*, *ngfr* and *abcg2*. The gene 18s served as the reference (housekeeping) gene to normalize expression data. The Ct values for each target gene were compared against 18s, and relative gene expression was calculated. Finally, the expression levels between the epithelial and cortical regions were compared for each stem cell marker.

Statistical analysis

To interpret the data, a significance level of 0.05 and a confidence interval of 95% were applied, taking into account the sample size used in the study.

Results

The expression of *nestin* in lens epithelial cells was found to be 229 times higher compared to that in lens cortical fiber cells. Similarly, *abcg2* showed a 23-fold increase in the epithelial region. In contrast, ngfr exhibited a modest increase, with expression levels 0.9 times higher in epithelial cells than in cortical fiber cells (Figure 1).

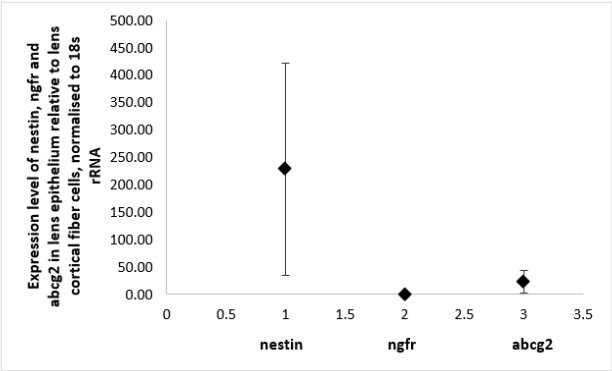

Figure 1. mRNA expression of lens stem cell markers *nestin*, *ngfr* and *abcg2* in the normal rat lens. Error bars are 95% confidence intervals for mean ratios in *nestin*, *ngfr* and *abcg2* mRNA expression between lens epithelial cells and lens cortical fiber cells.

Relative mRNA expression of the stem cell markers *nestin*, *ngfr* and *abcg2* in the normal rat lens. The error bars represent 95% confidence intervals for the average expression ratios between lens epithelial cells and cortical fiber cells.

Additional data showing the variation in Ct mean differences for *nestin, ngfr* and *abcg2* expression between the two lens regions are presented in Figures 2-4 respectively.

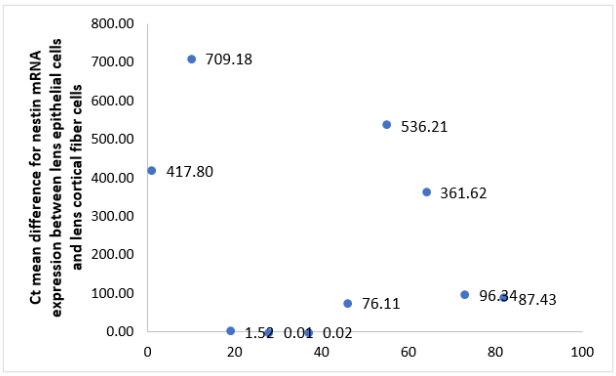

Figure 2. Dispersion of Ct mean difference of *nestin* mRNA expression between lens epithelial cells and lens cortical fiber cells.

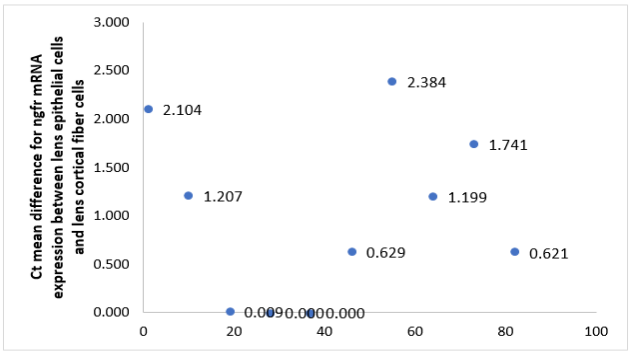

Figure 3. Dispersion of Ct mean difference of ngfr mRNA expression between lens epithelial cells and lens cortical fiber cells.

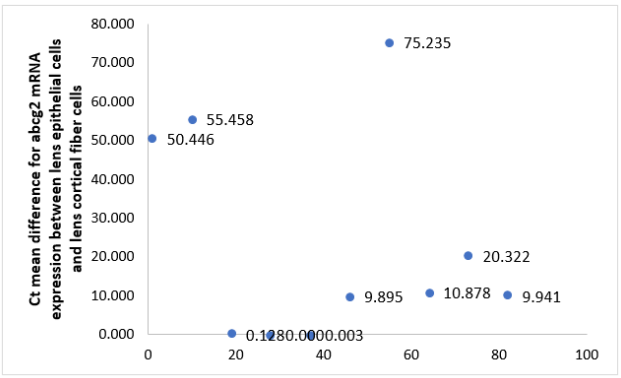

Figure 4. Dispersion of Ct mean difference of *abcg2* mRNA expression between lens epithelial cells and lens cortical fiber cells.

Discussion

The purpose of this research was to examine the expression levels of the stem cell markers *nestin*, *ngfr* and *abcg2* in the normal rat lens.

The results demonstrated a significant upregulation of *nestin* in lens epithelial cells, showing a 229 fold increase compared to cortical fiber cells. This outcome aligns with previous observations of *nestin* expression in the mouse lens.

Similarly, ngfr expression was slightly higher in epithelial cells, with a 0.9 fold increase relative to cortical cells, supporting earlier findings in mouse lens studies. In the case of *abcg2*, a 23-fold elevation was observed in epithelial cells, consistent with prior mouse data.

The elevated expression of these markers in lens epithelial cells indicates the existence of cells with stem cell-like characteristics in the normal rat lens. This observation is consistent with the results reported by Oka et al., who reported a stem cell population in the germinative zone of the mouse lens, strengthens the evidence that a similar population may exist in rats. However, further investigation is necessary to confirm the protein-level expression of these markers and to accurately localize stem cells within the rat lens.

Interestingly, some variation was observed: Three rats showed negligible differences in Ct values for *nestin*, *ngfr* and *abcg2* between epithelial and cortical cells (Figures 2-4). This may be attributed to natural biological variability among individual animals.

Conclusion

In summary, the study concludes that *nestin*, *ngfr* and *abcg2* are more highly expressed in the lens epithelial cells compared to the cortical fiber cells in normal rat lenses. These findings support the presence of stem cell characteristics in the epithelial region and suggest the need for additional studies to compare these results with those observed in other species, particularly mice.

Ethics Approval

All methods are reported in accordance with ARRIVE guidelines for the reporting of animal experiments. All animals were treated in accordance with the ARVO statement for the use of animals in ophthalmic and visual research. Ethical approval was obtained from the Uppsala Ethics Committee on Animal Experiments, protocol number C29/10.

Funding

Not available.

Competing Interests

None to declare.

Availability of Data and Materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Authors' Contribution

Konstantin Galichanin: Designed, performed the experiment, analyzed the data and wrote and revised the manuscript. KG is also the guarantor of this manuscript.

Consent for Publication

Not applicable.

Patient and Public Involvement

Patients or the public were not involved in the design or conduct or reporting or dissemination plans of our research.

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