ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 6, Issue, 8, pp.5744-5748, August, 2015

International Journal of Recent Scientific Research

RESEARCH ARTICLE

DNA SEQUENCING OF REGENERATED LENS UNDER THE INFLUENCE OF VITAMIN-A IN YOUNG SWISS ALBINO MICE

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ARTICLE INFO

Article History:

Received 2nd, July, 2015
Received in revised form 10th, July, 2015
Accepted 4th, August, 2015
Published online 28th, August, 2015

Key words:

bushing, pressing-in, stress, aluminium alloy, alloy steel.

ABSTRACT

The present study supports and prove previous finding that Vitamin A can induce and accelerates lens regeneration in pigmented epithelial cells (PECs) of dorsal iris in Swiss albino mice. In Lens regeneration, many scientist shown that Vitamin A induces the mitogenic activity which causes functional impairment of retinoid receptors and thereby inhibits the lens regeneration. The purpose of present study to *understanding the DNA base pair difference between normal lens and regenerated lens DNA. The work was mainly based on histological and molecular aspects of lens regeneration. The study concludes that* the base pairs of regenerated lens DNA and normal lens DNA were almost similar except these SNPs. There may be some mutation or aberration of DNA base pair alignment present in regenerated DNA base pair compare to normal DNA base pair.

INTRODUCTION

Lens regeneration provides a clear example of trans-differentiation of one differentiated cellular type having a distinctive pattern of metabolic activities to another cellular type, which is morphologically and biochemically distinct from the original. An abundant literature exists on lens regeneration in amphibians[1-5]. Lens regeneration from non-ocular tissue (dorsal iris) has been well documented in amphibians[6-9].Regeneration is a developmental process which occurs during post embryonic period. It is the ability of fully developed organism to replace lost part by growth or remodeling of somatic tissues. Regeneration involve all those fundamental processes including cell proliferation, cell movement, morphogenesis, histogenesis and growth which occur during ontogenetic development in embryonic and larval stages. But lens regeneration differs from general regenerative process rather it provides a clear example of "metaplasia" During lens regeneration there is a transformation of one differentiated cellular type, having a distinctive pattern of metabolic activities to another cellular type, which is morphologically different from original and which synthesized a different array of macromolecules. The process was to as "metaplasia" Colucci (1891)[10] had first described lens regeneration from the dorsal iris termed wolffianregeneration. lens regeneration is considered as example of trans differentiation. Trans differentiation is a process by which differentiated cells alter their identity to become other distinct cell type. When the lens of a newt is removed, the process of regeneration is initiated from the dorsal iris[11]. The pigment epithelial cells (PECs) from the dorsal iris proliferate, dedifferentiate, and then trans differentiate into lens cells[12]. PECs initiate DNA synthesis and eventually lose their characteristics of origin, such as pigmentation. At about 7–10 days post-lentectomy a small vesicle is formed at the tip of the dorsal iris[13] Cells in this vesicle then trans differentiate into lens cells and form the lens vesicle (10–15 days). Cells from the posterior part of the lens vesicle differentiate to form the lens fibers (15–20 days). Lens regeneration is complete by 25 days post-lentectomy[14].

In current study we have found difference in DNA base pair sequence of normal lens and regenerated lens under the influence of Vitamin A. We have use Specific Gene RXR alpha as a primer in PCR and DNA Sequencing technique for provide information regarding base pair similarities and difference. It's a computational method of bioinformatics

DNA isolated from regenerated and normal lens of swiss albino mice. With this DNA sequence we were finding the effect of retinoic acid (A derivatives of Vitamin A) on regeneration of lens in Swiss albino mice. When any two human genomes are compared side by side, they are 99.9% identical (Cooper *et al*., 1985). DNA base pair alignment having dissimilarities and similarities will be compared by SNP technique.

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MATERIAL AND METHOD- GENERAL

Lens Regeneration provides a good model for the study of trans-differentiation ability of Somatic Cells. For this purpose young Swiss Albino Mice were employed as experimental animals. Nutrition and healthy environment were provided to swiss albino mice for healthy growth. The present work was designed into two parts:-

Fig 1 Photograph showing rearing of mice colonies in plastic case

The experiments were carried out on newly born young swiss albino mice (2 days to 40 days) lentectomy was carried out on 50 animals under local anesthesia (2% xylocaine). A longitudinal slit was made in the cornea of the right eye under a stereoscopic binocular microscope. The complete intact lens along with lens capsule was extracted through the incision. Following the operation, 40 IU/ml solution of vitamin A was injected intra peritoneal (I.P) on alternate days. In the case of 25 operated animals where vitamin A was not given, served as the control group. In second part or project following steps were performed:-

1. Genomic DNA was isolated from Mice Lens samples (Control and Regenerated Lens) using GeneiPureTM Mammalian genomic DNA Purification kit (# 117304)

2. Using Gene specific primers ~54bp fragment of **rxr alpha danio rerio**gene was amplified using Taq DNA Polymerase.

PCR conditions: The generxralphadaniorerio was amplified using genespecificprimer. The sequences of the primers areas follow.

Forwardprimer:**5'-AATGCTTCTTTCTGCTTTCC-3'**
Reverseprimer:**5'-CTGAGAGGAGAGGATGTCAC-3'**

Step1 94^0C–5min
Step2 94^0C-30sec
Step3 58^0C-30secfor35cycles
Step4 72^0C-30sec
Step5 72^0C-10min

3. The PCR product was cloned into T vector (Instant ligation kit # 105611) and sequenced..
4. Sequence data was analyzed to detect the SNP in the gene.

RESULT AND ANALYSIS

Group A:- Control – The animals were not given any treatment after their lentectomy. Only sham injections were given on alternate days. 5-5 each animals were preserved in Bouins Solution on days 2,7,15,20 and 40 days after operation

Group B:- Treated – The animals of this group were given treatment of Vitamin A after their lentactomy on alternate day basis. 5-5 each Animals were preserved in Bouins Solution on day 2,7,15,20 and 40 days after operation. All preserved animals were used for histological examination for find out different stage of trans differentiation

Results and Observation of First part of experiment

Table Showing the percentile of regeneration of new lens in Swiss albino Mice under influence of Vitamin A.

Sr. no	Group	Regenerated Lens	Regenerated Lentoids	Non-Regenerated Case	Percentile of Regeneration
1	Gr. A : - Vitamin A Treated	34	4	2	85%
2	Gr. B :- Control group Non treated	Nil	7	33	17.50%

First, the cells are cuboidal and slightly taller in shape, then they began to elongated and enter in the lumen of vesicle. The lumen which contains the primary lens fibre nuclei began to differentiate in to the secondary lens fibers. At least, the nuclei of the secondary lens fibers progressively disappear

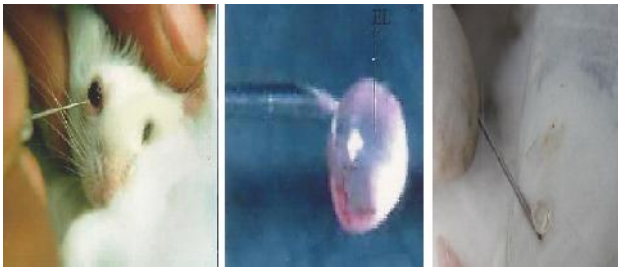
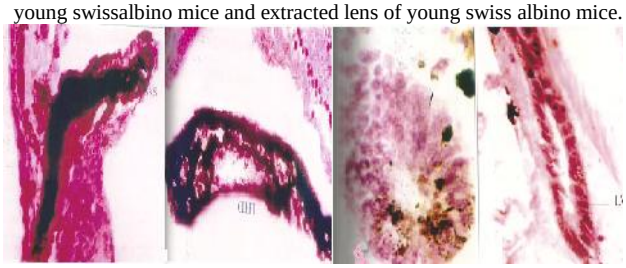
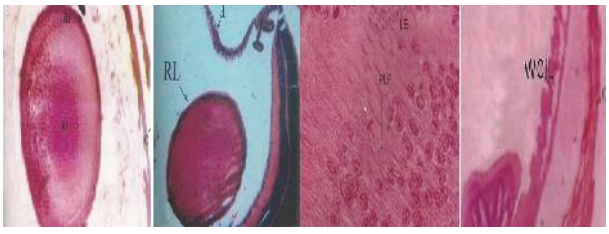


Fig 2 Microphotograph showing insertion of needle in to the right eye of young swissalbino mice and extracted lens of young swiss albino mice.



A B C D

Fig 3(A). Microphotograph of section through dorsal iris of vitamin A treated young swiss albino mice showing transdifferentiation of iris into lens cells. Pupillary margin of dorsal iris becomes swollen and knob like. **(B)** Section through dorsal iris showing the formation of initial lens vesicle. The central calls are transformed in to lens forming cells.**(C)** section through dorsal the eye showing well defined lens vesicle at the tip of dorsal iris. Mitotic figures are also visible in the epithelium. **(D)** Section through the dorsal iris of operated eye of vitamin A treated mice showing formation of lens vesicle at the tip of dorsal iris.



A B C D

Fig 4 (A). Microphotograph of section passing through the L.S. of vitamin A treated swiss albino mice regenerated lens. Section showing well differentiated lens with secondary lens fibers.(B). Section showing detached regenerated lens and it's position aling with dorsal iris and retina (C).Section passing through the regenerated lens of vitamin A treated young swiss albino mice showing differentiation of primary lens fibers. (D). Section passing through the operated eye of untreated control group of young swiss albino mice showing lens regenerated case with wavy and thick epithelium of iris

Isolated DNA was stored at -20° C in sterile vials with marking of vials. This isolated DNA sample was further used in PCR

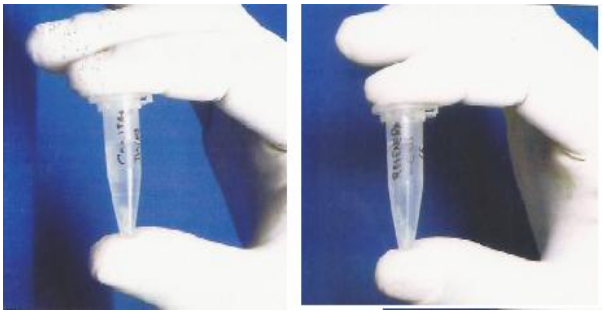
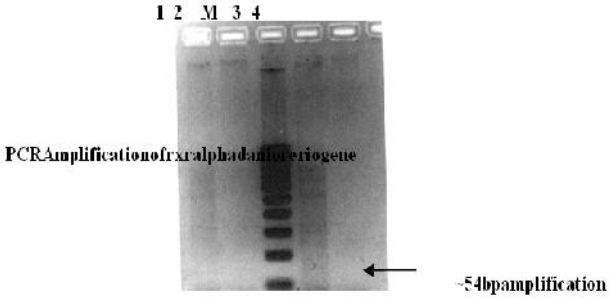


Fig 5 Microphotograph showing isolated lens DNA from control group (non-treated with vitamin A) and regenerated group (treated with vitamin A) of swiss albino mice in eppendrof.



Lane1-2: PCR amplification of Normal Lens
Lane M : StepUp100bpLadder(#118707)
Lane3-4: PCR amplification of Regenerated Lens

- The PCR products were loaded on 1.5% agarose gel.
- The amplified PCR products were Cloned into T vector
- Clones were confirmed digesting the plasmids with *NcoI* restriction enzyme.
- Positives Clones were sequenced with M13F primer (Vector specific primer)
- The sequencing data was studied for SNPs.

Sequencing Data

NormalLens#1

GATGTAATACGACTCACTATAGGGCGAATTGGGCC
CGACGTCGCATGCTCCCGGCCG**CCATGG**TTAATGC
TTCTTTCTGCTTTCCGCACGAGTGAGTGACATCCTCT
CCTCTCAGAT**CCATGG**CCGCGGGATATCACTAGTG
CGGCCGCCTGCAGGTCGACCATATGGGAGAGCTCC
CAACGCGTTGGATGCATAGCTTGAGTATTCTATAGT
GTCACCTAAATAGCTTGGCGTAATCATGGTCATAGC
TGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCC
ACACAACATACGAGCCGGAAGCATAAAG

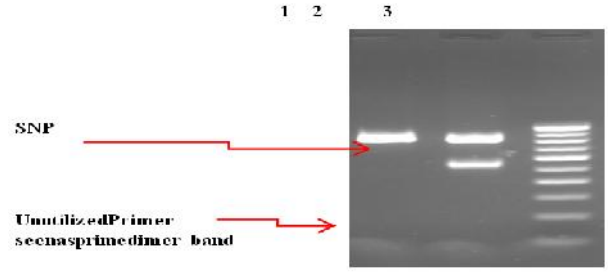
RegeneratedLens#2

CCGATGTATACGACTCACTATAGGGCGAATTGGGCC
CGACGTCGCATGCTCCCGGCCG**CCATGG**TTAATGC
TTCTTTCTGCTTTCCTCTCAGGTGACATCCTCTCCTC
TCAGAT**CCATGG**CCGCGGGATATCACTAGTGCGGC
CGCCTGCAGGTCGACCATATGGGAGAGCTCCCAAC
GCGTTGGATGCATAGCTTGAGTATTCTATAGTGTCA
CCTAAATAGCTTGGCGTAATCATGGTCATAGCTGTT
TCCTGTGTGAAATTGTTATCCGCTCACAATTCCACA
CAACATACGAGCCGGAAGCATAAAGTGT

Blue Indicates gene of interest

Red Indicates *Nco I* Restriction enzyme site

SNP presence in 1.5 % Agarose gel (Stained with EtBr) visible on transilluminator



Lane1:NormalDNA (Normal Lens DNA)
Lane2:SNP DNA (Regenerated Lens DNA)
Lane3: 100 bpDNALadder

Regenerated DNA gives 2 bands whereas amplification using normal DNA gives only one band. Hence it can be concluded that template #1 is normal and template # 2 is SNP type.

Subject sequence is Normal DNA sequence and Query sequence is regenerated lens DNA sequence. Blast technique confirms that if we usedGenespecificprimers~54bpfragmentofRXRalphadanio-reriogene. Then above mention SNP were produced. This type of SNP was came due to many reason like environment, handling and nutrition etc. But as according to our experiment and past research Vitamin A is major cause which is responsible for this type of mutation and SNP. This DNA mutation and SNP is also a cause of regeneration in Swiss albino mice.

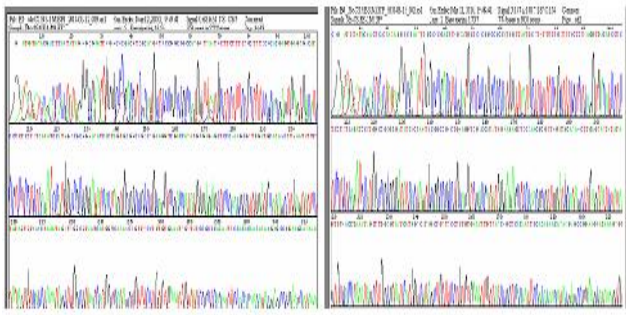
BLAST Alignment result of Normal Lens and Regenerated Lens:

>lcl|48827 Score=66.2bits(72), Length=50, Expect=2e-17, Identities=48/54(88%),Gaps=4/54(7%) Strand=Plus/Plus

```
Query  1   TTAATGCTTCTTTCTGCTTTCCGCACGAGTGAGTGACATCCTCTCCTCTCAGAT  54
           ||||||||||||||||||||||  | | ||  ||||||||||||||||||||||
Sbjct  1   TTAATGCTTCTTTCTGCTTTCCTCTC-AG---GTGACATCCTCTCCTCTCAGAT  50
```

The identified SNP's are G,A,G,T,G and A

Electropherogram: Fluorescently labeled DNA fragments were separated according to their molecular weight. All separated DNA base pair provide a peak as according to their properties these peak provide a colored graphically presentation



Normal Lens DNA **Regenerated Lens DNA**

DISCUSSION

In regeneration process histological study revealed that during lens regeneration after lensectomy the two layers of pigmented epithelium of the dorsal iris thickened and a cleft developed between two lamina of the dorsal iris (Figure 3 and the nuclei of iris cells changed their shape. Then the pupillary margin of the iris become knob-like. The formation of this knob- like structure continued until the free margin be came as wollen loop-like structure. Scattered mitotic figures were also observed. All these changes continue up today 7after operation in vitamin A treated animals. Then the cells started to dedifferentiate: they threw out their melanosomes. The semelanosomes are ingested by macrophages that entered from the wounded site. Dorsal iris cells continued to divide, forming a vesicle-like structure in the region of there moved lens. The vesicle differentiated into anew lens. Once the new lens formed, the cells of the dorsalirisceasedmitosis. The newly for medleys was surrounded by alensepithelium whose cells were cubiodal and slightly taller. Lens fiber formation was initiated in the inner surface of the vesicular lens. At that time cells elongated and entered the lumen of the vesicle. Gradually the lumen was filled by primary lensfibernuclei(Figure4).Lateron the second arylensfibers differentiated and grew around the central nucleus and the regenerated lens became a better-defined structure With the help of above result we can conclude that vitamin A is major responsible factor for *Trans-differentiation* in dorsal iris of swiss albino mice eye.

In DNA base pair study with the use of DNA isolation, PCR, DNA Cloning, DNA sequencing and Blast technique its concluded that **G,A,G,T,G and A** these SNP's are present in regenerated lens DNA sequence. This is proved in SNP detection test. In SNP test Regenerated DNA gives 2 bands where as amplification using normal DNA gives only one band. Hence it can be concluded that template #1 is normal and template # 2 is SNP type. This SNP is further concluded in BLAST comparison and Electopherogram. Now it's proved that vitamin A work as an inducer for dorsal iris and its show some mutation or aberration of DNA base pair alignment in regenerated DNA. Vitamin A is responsible for all these genetic changes.

Acknowledgement

We are thankful to Developmental Biology Lab,Dunger College, Bikaner for the experimental support Also, thankful to Plant Biotechnology, Centre, Rajasthan Agriculture University Bikaner and Bangalore Genei, Bangalore for providing infrastructure and equipment facilities.

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How to cite this article:

Vikram Singh Bhati *et al*., DNA Sequencing Of Regenerated Lens Under The Influence Of Vitamin-A In Young Swiss Albino Mice. *International Journal of Recent Scientific Research Vol. 6, Issue, 8, pp.5744-5748, August, 2015*

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