Abstract

The present study deals with the characterization of dense cataract and microphthalmia (dcm), a congenital eye abnormality in the BALB/c mice. The eye abnormality, which was spontaneously noticed with congenital cataract and microphthalmia on 14th postnatal day when eyes are open, found to be inheritable in nature by backcrossing study. The colony of this mutant mouse was established by full sib mating. The affected mice exhibited mild opacity earlier, progressed with the age as a dense cataract, looked like mature cataract and occupied lens completely at the age of 8 weeks. The genetic experiments using standard breeding protocols revealed a genetic defect transmitted through autosomal recessive inheritance. Based on the appearance of mutant phenotype, the mutant mouse model was designated as dense cataract and microphthalmia (dcm).

With the aim to study this eye abnormality in detail, dcm mouse model was extensively characterized for phenotypic, genetic, histological alterations, as well as identification of molecular lesion(s) and its relationship with the phenotype observed in the affected mice.

The clinical examination of mutant phenotypes carried out by unaided eye and also with the help of ophthalmoscope at various ages, revealed four major eye defects such as bilateral cataract, microphthalmia, microphakia and aniridia.

The mass measurements of mutant animals, eyes and lenses recorded at 0, 30, 60, 90 postnatal days and at 1 year age revealed that the body weights were not affected; however there was significant reduction in the weight and size of eyes and lenses. The decrease in eye and lens mass was noticed at all ages tested; irrespective of sex, revealing the significant impact of mutant gene(s) on postnatal growth of eyes and lenses except general body.

The embryonic eye histology revealed normal morphogenesis of eye during early eye development; however, morphology of primary lens fiber cells was altered on 13th
Embryonic day (E13) followed by progressive deterioration of the lens cell micro architecture during gestation and subsequent postnatal period leading to complete destruction of lens morphology.

The major lens crystallin proteins (LCP) from the lenses of *dcm* mutant mice were separated by 20% SDS-PAGE and compared with that of WT mice. The LCP from *dcm* mutants were separated normally between 20-30 kDa molecular weight range. No significant change was noticed in the distribution pattern of LCP when compared to wild-type (WT) mice; however, expression of LCP in *dcm* mutant mice was reduced as evidenced by low resolutions of protein bands. Immunoblotting analysis using anti-α, anti-β and anti-γ crystallin antibodies further identified the expression of major lens crystallin proteins, i.e. α, β and γ-crystallin proteins in *dcm* mutant lenses.

To identify defects in *dcm* lenses at molecular level we analyzed their proteome by performing high resolution 2-dimensional (2-D) polyacrylamide gel electrophoresis. The gel electrophoretic profile of *dcm* lenses was compared with that of WT lenses to determine if relative changes in crystallins could be detected. Relative increase and decrease in intensities of several protein spots were detected in the *dcm* mutant 2-D gel. The separated proteins spots included both WT and mutant crystallins proteins. Abundance of proteins between 4- 7 pI and absence of few protein spots in the range of isoelectric points (pI) 7-9 was distinct feature noticed in *dcm* mutant gel when compared with the WT.

One differentially expressed protein spot from *dcm* mutant and four spots from the WT 2-D gels were excised and subjected for protein sequencing by protein mass spectrometry. The sequence analysis of one spot from *dcm* mutant gel displayed high homology with αB Crytsallin, while wild-type protein spots between 7-9 isoelectric points exhibited homology with γD, γB, γC and γA-Crystallins. This finding indicated that the αB Crytsallins were upregulated whereas the subunits of γ-crystallins were deficient in *dcm* mutant lenses. It is possible that the over expression of αB Crytsallins as well as deficiency of γ-Crystallin cluster in *dcm* mutant lenses could be related with the
development of cataractogenesis in dcm phenotype. Further study is necessary to look into the mechanism that causes the upregulation of αB Crystallin and deficiency of γ-Crystallin subunits in dcm mutant lenses.

To understand whether the defect in the expression of γ-Crystallins is due to a mutation in coding sequence, genes encoding for γ-crystallins were amplified by polymerase chain reaction (PCR) and subjected for DNA sequence analysis. The amplified sequences of mutant mice were compared with WT sequences using BLAST. Sequence analysis of PCR products indicated that the genomic regions coding for the c & d subunits of Cryg were not mutated in dcm mutant mice. Thus, the defect could be in regulatory regions or at the level of transcription factors regulating the expression of these genes.

In light of these, efforts to pinpoint the molecular lesion(s) associated with one or more genes in dcm mutant mice will be highly useful to explore the pathobiology of this genetic disorder of the eye as well as to understand the mechanism of congenital eye defects in humans. Further study to locate the defective gene(s) on chromosomes by genome-wide linkage analysis using several microsatellite markers for each autosome is necessary.