Faculty, Biomedical

Effect of CARMA 2sh gene in Mouse embryonic stem (ES) cells

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BACKGROUND

- CARMA2 belongs to the CARMA family of proteins. They are involved in the regulation and activation of NF-κB, that have a central role in the control of immune and inflammatory response, and cell survival and proliferation.
- CARMA2short (CARMA2sh) which is the most prominent CARMA2 isoform expressed in human

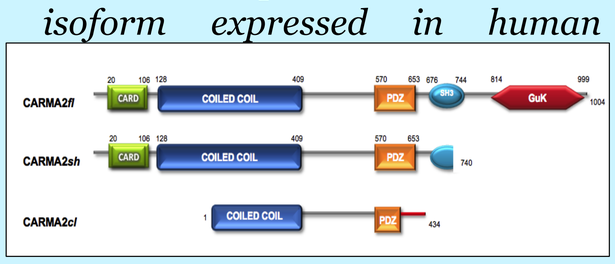

Fig.1 illustrates the three isoforms of CARMA 2

- It has already been identified that CARMA2sh induces activation of NF-κB, and this activity requires the function of another CARD-containing protein, namely BCL10, and the adapter protein TRAF2.
- This study identified a CARMA Inhibitory Kinase(CIK) which inhibit the ability to induce NF-κB.
- CIK is not tested for their function in Human Primary keartinocytes and hence we attempt to understand the function of CIK and its associated molecules by invitro and invivo models.
- The inhibitory activity of CIK on CARMA2 in primary human keratinocytes expressing wild (wt) & mutant CARMA2 was analyzed

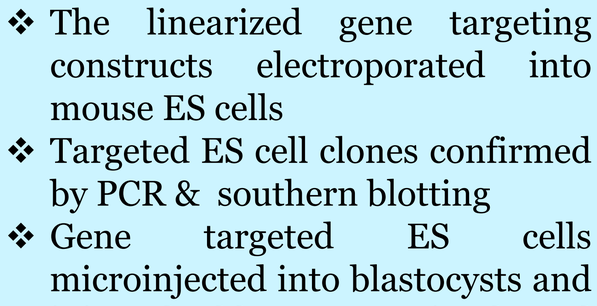

Fig.2 displays the CARMA 2 mutation in inflammatory disorders

AIMS

- Generation of CARMA2 mutant associated with psoriasis (Gly117Ser and Glu 138Ala) by site-directed mutagenesis.
- Designing targeting vectors with a selection marker & generating transgene via site - specific DNA recombination method.
- The linearized gene targeting constructs electroporated into mouse ES cells
- Targeted ES cell clones confirmed by PCR & southern blotting
- Gene targeted ES cells microinjected into blastocysts and injected blastocysts implanted into 10-15 pseudopregnant females.
- Chimeric litters will be then transferred for breeding.

EXPERIMENTS

Site directed mutagenesis

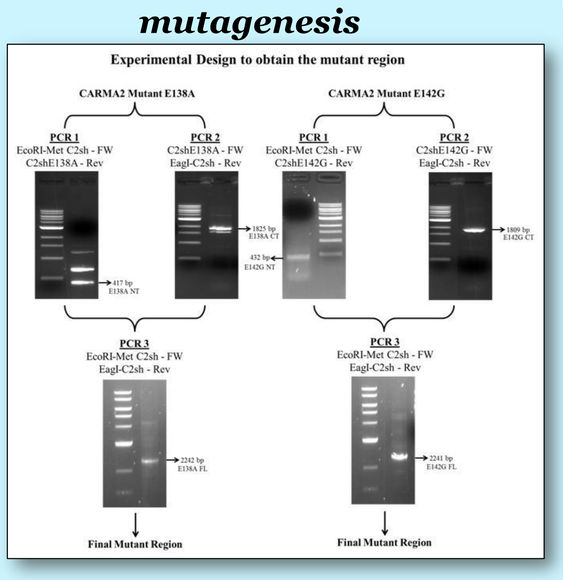

Fig 3 : schematic explanation of the generation of CARMA 2 mutants by site directed mutagenesis

Mutations were created through site directed mutagenesis method. The successful introduction of the mutations was confirmed by standard sequencing.

HEK culturing & Gene expression analysis

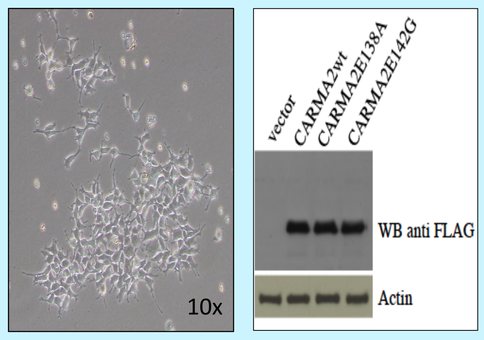

Fig 4: CARMA2wt and mutants expression in HEK293 cells.

HEK293 cells were transiently co-transfected with CARMA2wt and the psoriasis-linked mutants. After incubation, gene expression analysis done by western blotting method.

Generation of wild & mutant Rosa26 vectors

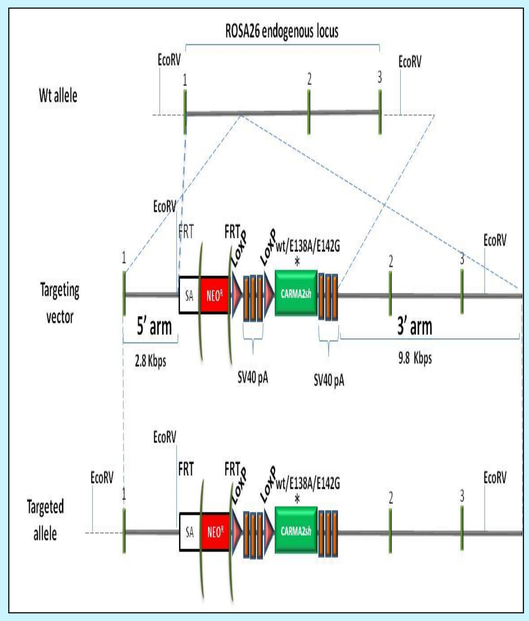

Fig 5 : Generation of murine strains expressing Hprt-Cre regulated CARMA2shE138A and CARMA2shE142G from the Rosa26 locus.

To generate the transgenic constructs, Rosa26-based vectors were used.

ES cell culturing & Southern blot analysis of transgenic clones

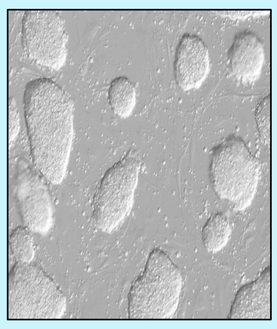

Fig 6 : Positive transgenic clones of ES cells

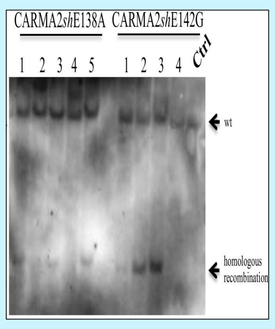

Fig 7:Southern blotting analysis of ES transgenic clones

ES cells were cultured in DMEM medium and incubated at 37°C & 5% CO_2. Selected wild & mutant vectors were electroporated in cultured ES cells & incubated at appropriate conditions. After incubation, selected clones were chosen for further study. Selected positive clones were confirmed by southern blotting

Generation of genetically modified mice

Stage I

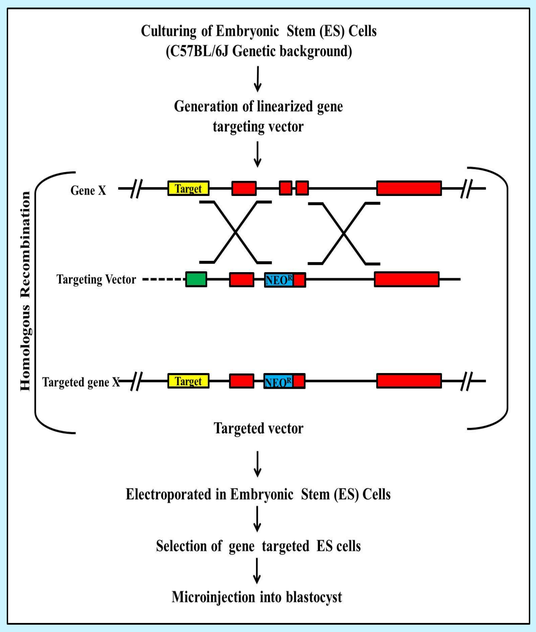

Fig 8: ES culturing and electroporation of target vector into ES cells

Stage II

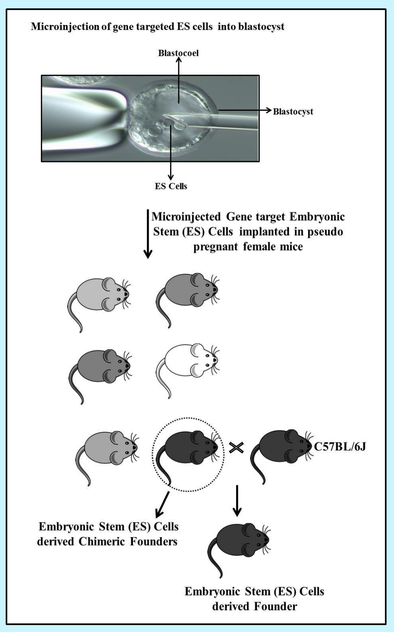

Fig 9: Microinjection and generation of knockout mice

Targeted ES positive clones were microinjected to the blastocyst. After microinjection and embryo transfer, the recipient female mice delivered & the pups were examined daily for any abnormalities. After 10th day, tissue skin biopsy (Tail or Ear) was taken from the pups and subjected to genotyping analysis to determine the transgenic founders

CONCLUSION

- We investigated the effect of CARMAsh RNA mediated knockdown CIK on the activation of NF-kB.
- This leads to reduction in the expression level of NF-kB target genes.
- CARMA2 depletion in HEK activates signal transduction pathways that control cell death and proliferation.

ACKNOWLEDGEMENT

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