Effect of psoriasis linked CARMA2sh gene in transgenic animal model

KAVITHA Varadharajan[1], MURALITHARAN Shanmugakonar[1]*, PASQUALE Vito[2]
[1]LARC, Qatar University, Qatar. [2]Molecular Division, Biogem, Italy.

Member of Qatar Foundation

Faculty and PostDoc, Medical, Biomedical and Health Science

Background

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

Fig.1 illustrates the three isoforms of CARMA 2

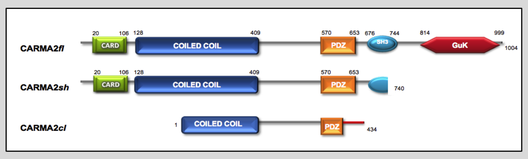
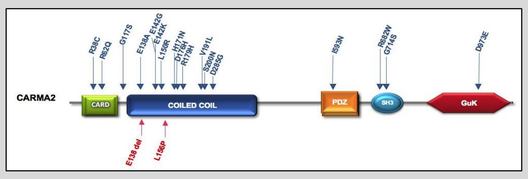


Fig.2 displays the CARMA 2 mutation in inflammatory disorders



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

Objective

- *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.*
- *Gene targeted ES cells microinjected into blastocysts and injected blastocysts implanted into 10-15 pseudopregnant females.*
- *Chimeric litters will be then transferred for breeding.*
- *Maintenance and observation of Transgenic animal*
- *Molecular analysis of transgene protein expression*

Methods

- ***Generation of wild & mutant Rosa26 vectors:***

The constructs were generated using standard molecular biology techniques and were adopted from collaborators laboratory, Biogem , Italy.

- ***ES cell culturing & analysis of 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***

Targeted ES positive clones were microinjected to the blastocyst. After microinjection and embryo transfer, the recipient female mice delivered & the pups were examined daily

- ***Phenotypical Analysis of transgenic mice***

Physical observation of transgenic animal and the keratinocyte proliferation rate were assayed

- ***Gene Expression and western blot analysis***

RNA & proteins were extracted for the expression analysis

Conclusion

- *We investigated the effect of CARMA2sh 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 transgenic cells activate signal transduction pathways that control cell death and proliferation.*

Results

Generation of genetically modified mice

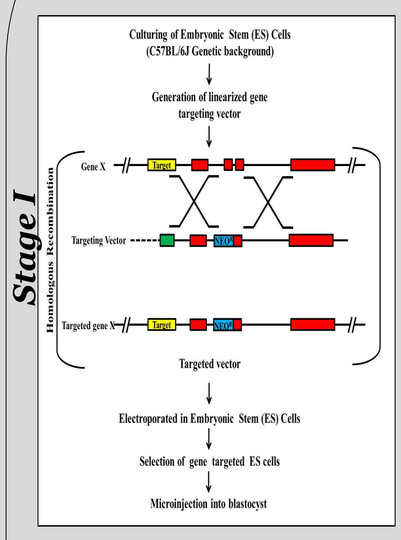


Fig 1: ES culturing and electroporation of target vector into ES cells

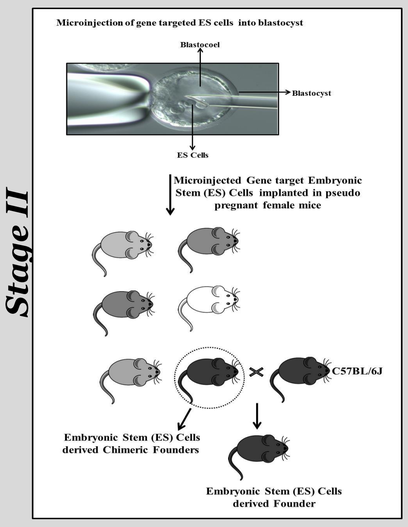
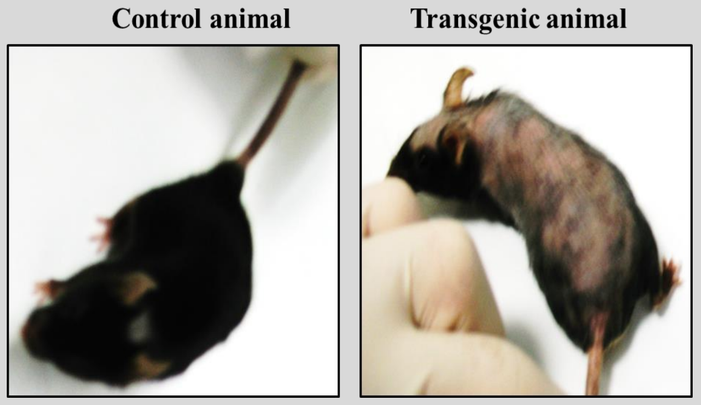


Fig 2: Microinjection and generation of knockout mice

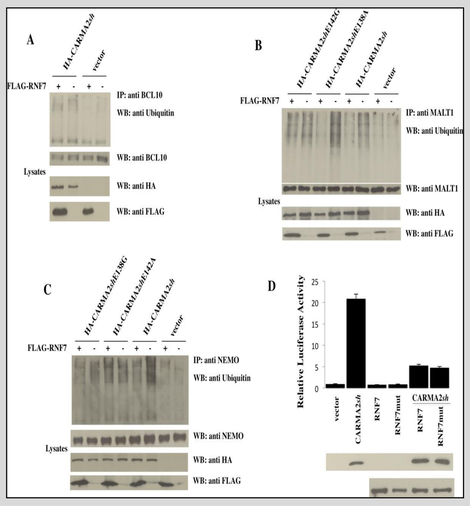
Targeted ES positive clones were microinjected in to the blastocyst. After microinjection and embryo transfer, the recipient female mice delivered the pups and were examined daily for any abnormalities. After 10th day, tissue skin biopsy was taken from the pups and subjected to genotyping analysis to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

Phenotypical Analysis of transgenic mice



Control and the CARMA2 mutant transgenic animal are shown in Figure 3. When compared to control animals, complete hair loss was observed on the dorsal side of the transgenic animals. This indicates that the animal expresses CARMA2sh mutation induced psoriasis.

Gene Expression and western blot analysis



- *RNF7 plays a key ubiquitination role in CBM mediated activation of NF-kB pathway. We tested whether RNF7 can influence such activity. .*
- *Expression of CARMA 2 associated RNF7 from transgenic keratinocytes induces the ubiquitination reaction of BCL10 (A) , MALT1 (B) and NEMO (C) using western blot analysis.*
- *The transfection of CARMA2sh in keratinocytes cells results in ubiquitination of BCL10 (Figure 4A)*
- *Expression of RNF7 doesn't show any inhibition with the ubiquitination reaction, whereas RNF7 reduces the ubiquitination of MALT1 & NEMO significantly which is induced by CARMA2 expression (Figure 6 B & C).*
- *Interestingly, RNF7 reduces the ubiquitination of MALT1 and NEMO induced by psoriasis-associated mutant CARMA2shE138A, but not that induced by psoriasis-associated mutant CARMA2shE142G. Figure 4D explains the luciferase activity of CARMA2sh and RNF7 control & mutant.*

ACKNOWLEDGEMENT *This work was made possible by NPRP grant NPRP 7 - 466 - 3119 from the Qatar National Research Fund of Qatar Foundation. Authors would like to acknowledge Biogem, Italy and LARC, Qatar University for their support.*