Expression of active and inactive forms of CIK and CARMA 2sh proteins in Normal Human Epidermal Keratinocytes (NHEK)

16-034

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ABSTRACT

Psoriasis is a debilitating skin disease affecting approximately 23% of human population. The disease is considered to have key genetic underpinning and genome wide association studies and meta analysis have identified more than 40 susceptibility loci for psoriasis. CARMA proteins play a major role in regulating activation of transcription factor NF-kB which is ubiquitously expressed in mammalian cells that play a central role in the control of immune and inflammatory response. Missense mutations in the CARMA2 gene have been shown to dominantly transmit the psoriatic trait with high penetrance. Most of the CARMA2 mutations associated with skin disorders clusters in the coiled coil domain of the protein.

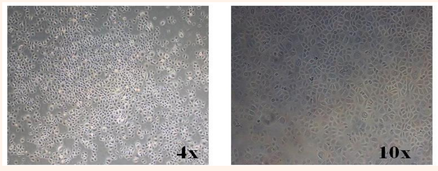
AIMS

- *Culturing of normal human epidermal keratinocytes (HEK)*
- *Preparation of expression constructs expressing active or inactive forms of CIK and CARMA2sh.*
- *Transformation of respective constructs & Transfection in HEK cells*
- *Expression level of target gene will be analyzed by western blotting.*
- *Reverse transcriptase-polymerase chain reaction analysis on NF-kB target genes*
- *The expression of CARMA associated proteins will be examined by immunohistochemistry*

RESULTS

HEK cell culture

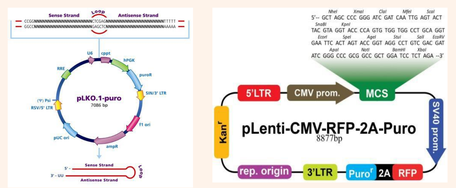
Fig.3 illustrates the microscopical view of HEK cells



- *HEK cells were cultured and after 3-5 days of incubation, the cells obtained confluent.*
- *The active cells were used for further transfection studies*
- *The virulent cells can also be stored in the Liquid nitrogen with cell freezing medium*

Construct

Fig 4 Schematic representation of the constructs generated.

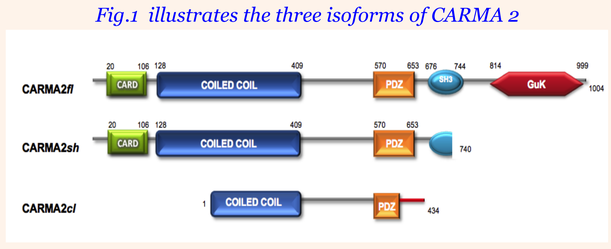


- *Standard lentiviral backbone for cloning and expression of new shRNA sequences.*
- *Standard cloning techniques were followed to construct the respective active & inactive gene forms.*

BACKGROUND

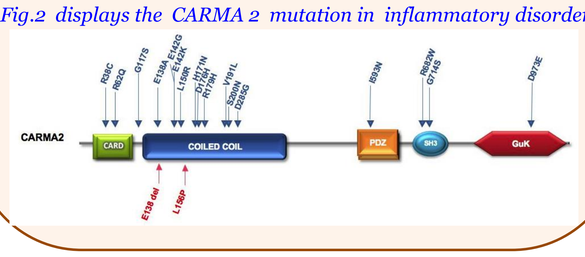
- *CARMA2 belongs to the CARMA family of proteins. They are involved in the regulation of activation of NF-kB, that have a central role in the control of immune and inflammatory response, and in the regulation of cell survival and proliferation.*
- *CARMA2short (CARMA2sh) which is the most prominent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

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.*
- *Interestingly, there is a recent finding of novel CARMA Inhibitory Kinase(CIK) which inhibit the ability to induce NF-kB.*
- *However these molecules are not tested for their function in Human Primary keratinocytes and hence in our project we attempt to understand the function of CIK and its associated molecules by invitro and invivo models.*
- *Further investigate on the inhibitory activity exert by CIK on CARMA2 in primary human keratinocytes expressing wild (wt) & psoriasis-associated mutant.*

Fig.2 displays the CARMA 2 mutation in inflammatory disorders



METHODS

HEK cell culture:

- *HEK was obtained from public Health England.*
- *Cells were cultured in Keratinocyte growth medium and incubated at 37°C under 5% CO2*

Construct :

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

Transformation:

- *The construct expressing active and inactive forms of CIK and CARMA2sh were made and transformed in E.coli DH5α.*
- *Transformation of the respective construct was done by standard protocol and the E.coli was cultured in LB broth.*
- *After incubation, the plasmid was extracted from the E.coli by commercially available Kit method.*

Transfection :

- *The plasmid vectors containing active and inactive forms were transfected in human epidermal keratinocyte by DreamFect Gold kit method.*
- *After incubation, the transfected cells were observed under fluorescence microscope to check the transfection efficiency*

Expression Studies :

- *After transfection, HEK cells were subjected for RNA & protein extraction (Trizol) & for immune staining analysis.*
- *The extracted RNA and protein were quantified and stored at – 80° C for further analysis.*
- *The cDNA was constructed from the extracted RNA for the gene expression analysis of NF-kB target genes.*
- *Immuno blotting analyses were done for the protein expression.*

Transformation

- *Target gene of interest were chosen & insert in a expression vector*
- *Vector construct was incorporated into the competent cells*
- *Cloned bacterial cells were cultured and plasmids were extracted*

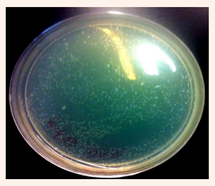
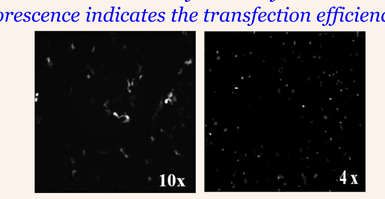


Fig. 5 shows transformed cloned bacterial cells .

- *Respective cloned vector were transfected in HEK cells*

Transfection

Fig. 6 shows HEK cells after transfection and the fluorescence indicates the transfection efficiency.



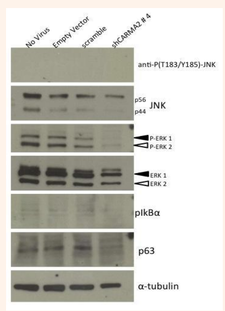
- *After Transfection, HEK cells were observed under fluorescence microscope to check the transfection efficacy*
- *After Transfection, HEK cells were subjected to Cell viability assay, RNA & Protein extraction for immuno blotting & staining analysis.*

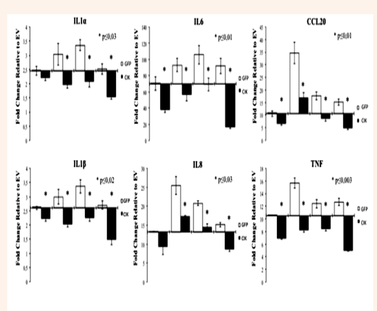
Expression Studies

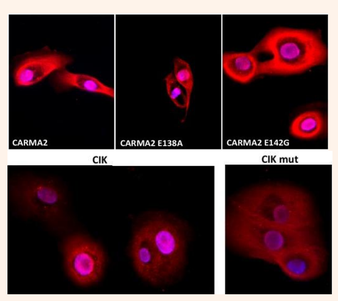
Fig.7 depicts the immunoblot assay of NHEK protein lysates

Fig.8 displays gene expression analysis of NF-κB target genes

Fig.9 depicts the immunostaining analysis of normal & mutant NHEK cells







- *The immuno blot analysis of normal and mutant forms of HEK cells protein lysates. It explains the reduced level of phospho kinase which involved in the post translation modification of cells*
- *RT –PCR Gene expression analysis explains the reduced expression level NF-κB target genes. These genes are involved in the inflammatory response of the particular target*
- *Immunostaining analysis revealed morphological variation of normal and mutant HEK cells*

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 *This work was made possible by NPRP grant NPRP 7 - 466 - 3119 from the Qatar National Research Fund of Qatar Foundation*