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.

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, Biogen, Italy.
- ES cell culturing & analysis of transgenic clones
  ES cells were cultured in DMEM medium and incubated at 37°C & 5% CO₂. 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-κB.
- This leads to reduction in the expression level of NF-κB target genes.
- CARMA2 depletion in transgenic cells activate signal transduction pathways that control cell death and proliferation.

Results

- It has already been identified that CARMA2sh induces activation of NF-xB 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-xB.
- 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 invito & invivo models.
- The inhibitory activity of CIK on CARMA2 in primary human keratinocytes expressing wild (wt) & mutant CARMA2 was analyzed

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