**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-xB, that have a central role in the control of immune and inflammatory response, and cell survival and proliferation.
- CARMA2sh (CARMA2sh) which is the most prominent CARMA2 isoform expressed in human keratinocytes.

**AIMS**

- Generation of CARMA2 mutant associated with psoriasis (Gly117Ser and Glu138Ala) 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**

**HEK culturing & Gene expression analysis**

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

**Generation of wild & mutant Rosa26 vectors**

- 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 genetically modified mice**

- Fig 5: Generation of murine strains expressing Hprt-Cre regulated CARMA2wtE138A and CARMA2wtE142G from the Rosa26 locus. To generate the transgenic constructs, Rosa26-based vectors were used.

**ES cell culturing & Southern blot 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.

**CONCLUSION**

- We investigated the effect of CARMA2sh RNA mediated knockdown CIK on the activation of NF-xB.
- This leads to reduction in the expression level of NF-xB target genes.
- CARMA2 depletion in HEK activates signal transduction pathways that control cell death and proliferation.

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