Human embryonic kidney cells

Health & Medicine

In figure A: Human embryonic kidney cells were transfected with constructs for carrying out this experiment. Immuno-precipitation was carried out of tagged PAG with an antibody against regulator Myc, its interaction is studied here with SRC & BRK follwed by immunoblotting analyzation. The result of this experiment was delaying in electrophorectic mobility of tagged PAG protein, when it was co-expressed with SRC. This delay is considered due to hyper-phosphorylation. However, on co-expression of PAG and BRK this band shift was less evident.

In figure C: Co-transfection of HEK 293 cells with MYC-PAG and SRC followed by treatment in the absence or presence of SRC kinase inhibitor SU6656 (5 M) for atleast 1 h. PAG was immunoprecipitated by antibody against MYC, and the binding of CSK was compared by immunoblotting.

The disturbance of link b/w CSK and PAG was observed on weakening SRC activity by a small molecular inhibitor SU6656, this experiment illustrated the importance of SRC kinase activity for functioning of CSK. B) to test the same hypothesis with controls, take breast cancer frozen samples of different patients, divide the sample in two groups one will be the test group other the control group. The control group will be given doses of anti tumor medication (say tamoxifen for breast cancer).

RNA will be extracted from both groups using trizol and will be followed by purification assay. The breast cancer cell lines will be transfected with empty vectors or pcDNA3-MKP3-V5. Further the transfectants will be placed in media MEM along with phenol red. Followed by SDS PAGE electrophoresis, and transfer to nitrocellulose membrane.

After transferring they will be incubated with primary antibody for an hour or above then with secondary antibody to observe the chemiluminescence with a reagent. The cells will then be lysed in a buffer, phosphatase reaction carried out will be observed via assays and transfected cells will be compared with control ones to whom tamoxifen was given.