

Elucidation of new binding partners of Tuberous Sclerosis Complex (TSC)

de Gemmis Paola, Hladnik Uros, Dolcetta Diego, Fortunati Elisabetta

Medical Genetics Unit - "Mauro Baschirotto" Institute for Rare Diseases - Costozza di Longare (VI) – Italy

E-mail: elisabetta.fortunati@birdfoundation.org



B.I.R.D. Foundation
Via. B. Bizio 1
Costozza di Longare (VI)
Tel. +39044455557
Fax: +39044455034

BACKGROUND

- Tuberous sclerosis (TSC) is an autosomal dominant disorder
- It is characterized by the widespread development of hamartomatous growth in many tissues and organs.
- It affects brain, kidneys, heart, eyes and lungs. Patients affected by the disease suffer from epilepsy, mental retardation, skin lesions, and benign tumors in various organs (Fig 1A).
- Mutations in TSC1 and TSC2 genes affect a complex mechanism of control of cell proliferation and differentiation via the mammalian target of rapamycin (mTOR) (Fig 1B).
- Mutations in genes other than TSC complex might influence the phenotype of patients.

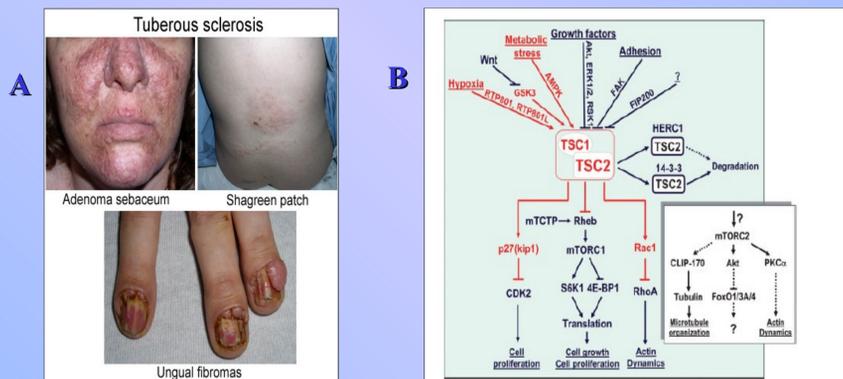


Fig 1 A shows the typical clinical signs of TSC patients. <http://www.uwo.ca/cns/resident/piclib.html>

Fig. 1B. Schematic representation of TSC1/TSC2 signaling. (Krymskaya et al., JBC 2008)

HYPOTHESIS AND PURPOSE

The heterogeneity of the TSC disease is a very well known phenomenon. Individuals of a family carrying the same genetic alteration might show a variable phenotype. This could be explained by mutations in genes other than TSC1 and TSC2 that affect the TSC complex and therefore influence the phenotype of patients.

The purpose of our study was the identification of new interacting binding partners for TSC2.

In the attempt to identify other genes interacting with TSC complex and modulating the disease expression we performed a yeast two hybrid screen using TSC2 as bait.

MATERIALS AND METHODS

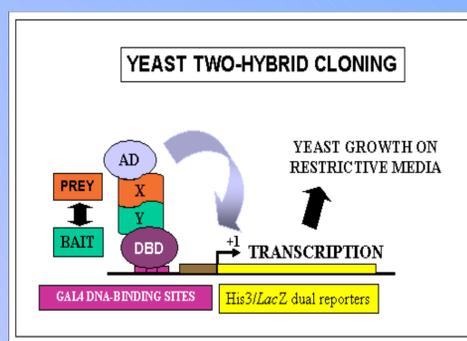
The full length TSC2 cDNA cloned as a 5.5 kb BamHI fragment into the pGBT9 yeast two hybrid vector (Clontech) was kindly provided by Dr. M. Nellist (Dept. Clinical Genetics, Erasmus Medical Center Rotterdam, the Netherlands). The sequence of the bait construct (pGBT9/TSC2) was verified by enzyme restriction digestion and sequencing, and the construct was introduced into AH109 (GAL4/2H-3) yeast cells (Clontech) by using a lithium acetate transformation protocol.

The transformants were used to screen a human liver pretransformed MATCHMAKER GAL4 cDNA library ($>1 \times 10^6$ independent clones [Clontech]) according to the manufacturer's protocol.

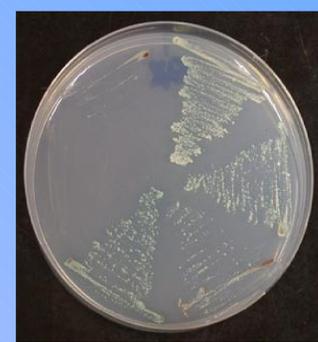
Briefly, AH109 [pGBT9/TSC2] cells were mated with Y189 yeast cells containing the pGADT7 library plasmids were selected by plating them on SD medium lacking adenine, histidine, leucine and tryptophan (SD/Ade/His/Leu/ Trp). Expression of proteins encoded by the library vectors was induced by growing the cells in the presence of galactose (SD/Ade/His/Trp medium [Clontech]). Surviving colonies were collected and re-plated on the same medium. Regrew colonies were further tested for β -galactosidase activity using β -gal assay according to the manufacturer's protocol. Positive yeast colonies, as indicated by activation of both reporter genes (His and LacZ), were independently identified and isolated. Plasmids were isolated from positive yeast cells by a glass beads/SDS 0.25% extraction protocol and the inserted genes were amplified by PCR and identified by direct sequencing. Obtained sequenced were searched for homologous gene with NCBI BLAST program.

RESULTS

- In an attempt to uncover new binding partners of TSC2 we used the full length cDNA of human TSC2 as bait to screen a human liver cDNA library containing 5×10^7 colony forming units.
- We selected 62 colonies growing in selective medium deprived of adenine, histidine, leucine and tryptophan.
- Among them, 13 colonies were positive for beta-galactosidase assay.
- We isolated plasmidic DNA from all 62 growing colonies and DNA was sequenced and blasted to NCBI database for gene identification.
- 2 out of 62 colonies contained in frame inserts encoding for the eukariotic elongation factor 2 (eEF2, NM_001961) and 60 S ribosomal protein L17 (NM_000985).

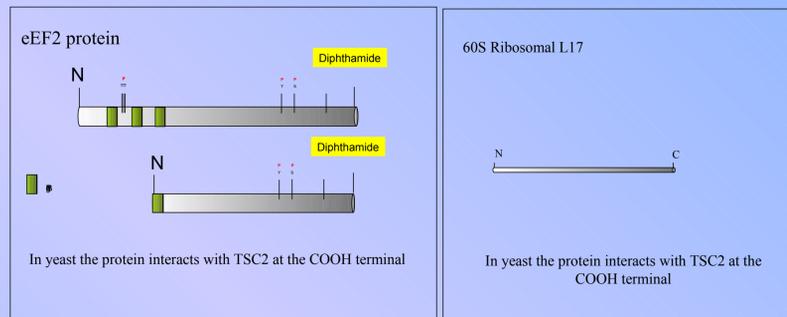


Principle of yeast two hybrid system



Retesting of positive colonies on selective medium

DISCUSSION AND FUTURE PLANS



Two specific TSC2 binding proteins were found in the screen analysis performed in yeast: the eukariotic elongation factor 2 (eEF2), implicated in the protein synthesis, and the 60S ribosomal protein L17. The phosphorylation of eEF2 is known to be dependent on mTOR which is the target of the TSC2 protein activity. The newly discovered TSC2-eEF2 interaction might shed light on new aspects of the protein synthesis regulation exerted by TSC2-mTOR pathway.

At the present, there are no data showing the involvement of L17 in the mTOR signalling pathways and/or in the TSC2 binding.

We are currently investigate the role of the identified proteins in the TSC2-mTOR pathway in mammalian cells and their regulation in the Tuberous Sclerosis.

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