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· The Role of the TSC1/2 Complex in Tumor Development

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AWARDS

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Over the last several decades, the study of hereditary tumor syndromes has laid a solid foundation for the genetic basis of cancer. While the number of patients suffering from these syndromes is small, the identification and elucidation of the underlying genetic pathways have shown to be of broad relevance to many forms of sporadic human cancers.

Investigations have found that the majority of hereditary tumors involve mutations of certain tumor suppressor genes. This latter class of genes has diverse functions including cell cycle regulation, DNA repair, apoptosis, protein degradation, cell-cell interaction, and signal transduction. However, a common feature of these genes is the "two-hit" genetic mechanism to inactivate their function during tumorigenesis. In the case of hereditary cancers, the first hit is inherited as a germline mutation of one of the alleles of the tumor suppressor gene, and the second hit is an acquired somatic mutation of the remaining allele of the same gene. This results in the loss of function of the tumor suppressor, thus creating a setting to promote tumor development.

One of the latest examples comes from the study of the tuberous sclerosis complex (TSC), an autosomal dominant disorder affecting more than 50,000 Americans. As a member of the phakomatoses, TSC is characterized by the appearance of benign tumors involving many organ systems, most notably the central nervous system, kidney, heart, lung, and skin. While classically described as "hamartomas," the pathology of the lesions is diverse, with features of abnormal cellular proliferation, growth (size), differentiation and migration.

Occasionally, TSC tumors progress to become malignant lesions (i.e., renal cell carcinoma). The genetic basis of this disease has been attributed to mutations in one of two unlinked genes, *TSC1* and *TSC2*. The protein products of these genes are found to negatively regulate the mTOR pathway, which controls protein synthesis, among other functions. Many human cancers have been found to exhibit abnormal activation of the PI3K/Akt/mTOR pathway, and recent clinical studies showed a therapeutic advantage in patients treated with an mTOR inhibitor. The key areas of current investigation focus on the elucidation of the molecular mechanisms of mTOR-related tumorigenesis, and the involvement of this pathway in liver cancer.

Growth Factor and Energy Metabolism in TSC Tumors

Studies in *Drosophila* have revealed a novel role of hamartin and tuberin in the PI3K/mTOR signaling pathway that is pivotal to the cellular response to growth factors (e.g., insulin) and nutrients. Genetic screens in mosaic flies for cell size control identified loss-of-function mutants of the *Drosophila* homologs of TSC1 and TSC2 that exhibit increased cell size in a cell-autonomous fashion. Conversely, over-expression of dTSC1 and dTSC2, but neither alone, effectively rescued this phenotype (i.e., reduced cell size). Genetic epistatic experiments in flies showed that the effects of dTSC1 and dTSC2 were dominant over dInR and dAkt, but not dTor and dS6K. Biochemical studies confirmed a negative regulatory role of the hamartin-tuberin complex in mTOR-dependent protein synthesis.

If indeed hamartin and tuberin act on distinct molecular targets in various pathways, how may their function be regulated?

The current model suggests that tuberin inhibits mTOR activity by serving as a GTPase activating protein for Rheb, a Ras-related protein, and consequently reduces p70S6K and 4E-BP1-dependent protein translation (Figure 1). Upon growth factor stimulation of PI3K, downstream activation of Akt results in phosphorylation of tuberin and releases its inhibition on mTOR. In TSC tumors, cells have lost TSC1 or TSC2 activity, thus resulting in uninhibited cell growth associated with elevated levels of mTOR and p70S6K activities. Indeed, pharmacologic blockade of mTOR with rapamycin, an immunosuppressant drug, causes profound anti-tumor response *in vivo*. However, it is not currently known how up-regulation of mTOR results in tumor formation, nor do we understand the mechanisms of tumor response to rapamycin.

Other unanswered questions include the physiologic role of TSC1/TSC2 in cellular metabolism, the function of the PI3K/mTOR pathway in tumor initiation, and the long-term efficacy of rapamycin in TSC pathology. These issues are being addressed using various cellular and *in vivo* models of TSC.

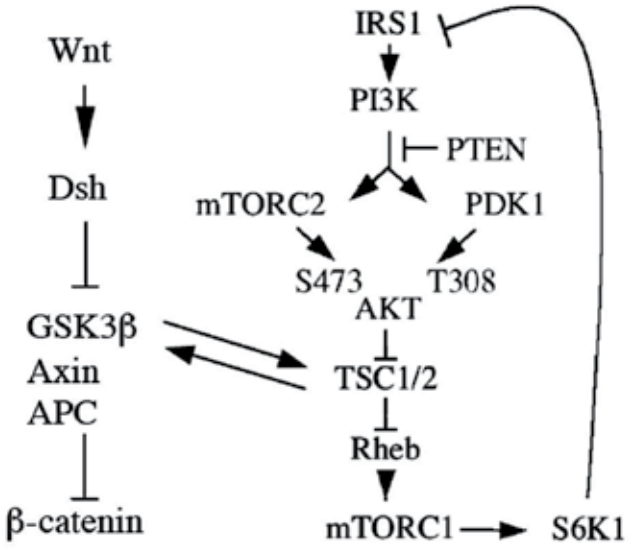

FIGURE 1: Model of TSC1/TSC2 pathway

The β–catenin Pathway and the TSC Genes

At present, not all of the TSC phenotype can be explained by one pathway. Our lab has explored the role of the TSC genes in the Wnt/β–catenin pathway. The latter has been implicated in the regulation of cell proliferation, differentiation, and migration. The Wnt family of secreted growth factors acts on multiple signaling cascades, among which the β–catenin canonical pathway is best understood for its role in various human cancers (e.g., colon, skin, liver). β-catenin is a highly conserved 95-kD protein involved in cell-cell adhesion and intracellular signaling. In its latter role, β-catenin shuttles from the cytosol to the nucleus upon Wnt stimulation, where it binds the LEF/Tcf family of transcription factors to activate downstream target genes such as cyclin D1 (Figure 1).

Our observations showed that renal tumors derived from our TSC animal model expressed high levels of β–catenin and cyclin D1. In 293T renal epithelial cells, expression of TSC1 and TSC2 reduced β–catenin levels by promoting its degradation. Correspondingly, TSC1/TSC2 inhibited β–catenin dependent activity of the LEF/Tcf transcription factors. Evidence suggested that TSC1 and TSC2 act at the level of the β–catenin degradation complex by associating with its components (i.e., GSK3, Axin) in a Wnt-dependent manner. Collectively, the TSC proteins likely function in multiple pathways, giving rise to the diverse manifestations of the pathology resulting from their inactivation (Figure 1). Efforts to demonstrate in vivo participation of these pathways and their relative contribution to the disease phenotype are currently our focus of investigation.

The Role of TSC1/2 in Microtubule Organization and Function

If indeed hamartin and tuberin act on distinct molecular targets in various pathways, how may their function be regulated? One possible mechanism for separating multiple activities within the cell could be on the basis of unique subcellular localization of the proteins. Since signaling complexes function as modules, the context in which they interact with other proteins depends on their localization. For example, insulin stimulation of PI3K leads to localized increased concentration of PIP3 at the plasma membrane. This, in turn, recruits Akt from the cytosol to the membrane, where it becomes activated.

In studying the subcellular localization of hamartin and tuberin, we found that they indeed reside in multiple compartments (i.e., cytosol, microsome, cytoskeleton). Of particular interest is the vesicular component in which tuberin was previously shown to interact with rabaptin-5 to modulate endocytosis. Biochemical analyses showed that the microsomal fraction of TSC2 belongs to the lipid raft domains and interacts with caveolin-1, a cholesterol-binding, structural protein of caveolae. Cells devoid of tuberin have mis-localized caveolin-1 and reduced formation of caveolae at the plasma membrane.

Recent studies point to a role of tuberin in regulating the transport of proteins such as caveolin-1 from the Golgi apparatus to the membrane. The molecular mechanism mediating this function of tuberin and the consequence of faulty protein trafficking in tumorigenesis remain to be elucidated.

Genetic Modifiers and Phenotypic Heterogeneity

One of the unexplained observations of the TSC syndrome is the variability in disease severity. This so called phenotypic heterogeneity can be seen in related individuals carrying the same genetic mutations, thus implicating the presence of other modifying factors.

Using animal models of TSC, we studied the influence of genetic background on tumor size and found that a specific TSC2 mutation, when placed into two unrelated strains of rats, produced vastly different disease burdens. By means of quantitative trait analysis, a genetic modifier was identified and mapped to rat chromosome 3.

It appears that this locus affects tumor size without significant influence on tumor multiplicity, suggesting a role in tumor progression rather than initiation. The identity of this gene and its function are currently being sought.

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