Molecular Mechanisms and Therapeutics of Skin Hamartomas in Tuberous Sclerosis Complex

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Tuberous sclerosis complex (TSC) is an autosomal dominant disorder with an incidence of one in 6000–10,000 live births [1]. Patients with TSC are predisposed to developing tumors (hamartomas) in the brain, eyes, heart, kidneys, lung, and skin. Although typically benign, these tumors cause significant morbidity, including seizures, cognitive impairment, and disfigurement. TSC is caused by mutations of a tumor suppressor gene, either TSC1 or TSC2. The proteins encoded by these genes, TSC1 (hamartin) and TSC2 (tuberin), function as a complex to regulate the mammalian target of rapamycin (mTOR) signaling pathway [2]. Loss of function of the hamartin-tuberin complex in TSC tumors enhances mTOR signaling leading to increased cell numbers and cell size. However, the molecular and cellular mechanisms of TSC tumor formation have not been fully elucidated.

Skin hamartomas, including multiple facial angiofibromas, ungual fibromas, forehead plaques and shagreen patches, are observed in about 90% of patients with TSC [3]. Histologically, these tumors show increased vessels and fibrosis, and variable but sometimes pronounced epidermal hyperplasia. Our studies focus on paracrine growth factors underlying formation of TSC skin hamartomas to identify new targets for treatment of TSC disease.

Angiogenesis in TSC skin hamartomas

Many TSC hamartomas have increased blood vessels, including tumors of the brain, kidney and skin [4]. These vessels are reactive and not neoplastic in most TSC tumors, including skin hamartomas [5]. Angiomyolipomas (AMLs) have reactive and neoplastic endothelial cells [6]. Tumors in animal models of TSC are also highly vascular [7, 8]. Increased vascularity may be due to release of many soluble factors by neoplastic cells, notably vascular endothelial growth factor A (VEGF-A, commonly called VEGF). Loss of TSC1/TSC2 function is accompanied by increased production of VEGF [9, 10] and this is related to mTOR activation and increased levels of hypoxia-inducible factor (HIF), a transcription factor that regulates the expression of VEGFA and other genes [7, 11]. We found that VEGFA levels were only modestly increased in TSC skin tumors [12]. Therefore, we sought to identify other paracrine factors or cell populations that might account for the dramatically increased vascularity of these tumors.

MCP-1 and macrophages in TSC skin hamartomas

Histologically, angiofibromas and periungual fibromas show increased numbers of fibroblast-like cells in the interstitial dermis together with mononuclear phagocytes, based on immunoreactivity for factor XIIIa [13-15]. Macrophages might be important for angiogenesis in TSC skin tumors and other TSC tumors as well. Increased numbers of cells positive for factor XIIIa have been observed in subependymal giant cell astrocytomas and angiomyolipomas [13]. Cortical tubers have been reported to contain increased numbers of cells expressing CD68, a marker of the monocytes/macrophages [16]. The factor(s) responsible for the presence of these cells in TSC skin tumors was unknown. To identify soluble factors with potential roles in TSC tumorigenesis, we screened TSC skin tumor-derived cells for altered gene and protein expression using a human cytokine/receptor gene array, real-time PCR and a multiplexed ELISA. Fibroblast-like cells from angiofibromas and periungual fibromas produced high levels of monocyte chemoattractant protein-1 (MCP-1) mRNA and protein, compared with those of TSC fibroblasts from the normal appearing skin of some patient [12]. We also found that the conditioned medium from angiofibroma cells stimulated chemotaxis of a human monocytic cell line more than did that from TSC fibroblasts, an effect blocked by neutralizing MCP-1 antibody. Our studies indicated that the over expressed MCP-1, by stimulating angiogenesis, fibrogenesis and recruiting monocytic cells, may play an important role in TSC tumorigenesis and offer a new therapeutic target.

Epiregulin mediates mesenchymal-epithelial interactions in TSC hamartomas

Patients with TSC develop hamartomas containing biallelic inactivating mutations in either TSC1 or TSC2. We compared TSC skin hamartomas to normal-appearing skin of the same patient, and observed more proliferation and mTOR activation in hamartoma epidermis, but “two-hit” cells were not detected.
in the epidermis. Fibroblast-like cells in the dermis, however, exhibited allelic deletion of TSC2, in both touch preparations of fresh tumor samples and cells grown from TSC skin hamartomas, suggesting that increased epidermal proliferation and mTOR activation were not caused by second-hit mutations in the keratinocytes but by mesenchymal-epithelial interactions. Gene expression arrays, used to identify potential paracrine factors released by mesenchymal cells, revealed more epiregulin mRNA in fibroblast-like angiofibroma and periangual fibroma cells than in fibroblasts from normal-appearing skin of the same patient [17]. Epiregulin is a member of the EGF family that includes EGF, transforming growth factor-alpha, heparin-binding EGF-like growth factor, amphiregulin, and betacellulin. Epiregulin is an autocrine or paracrine factor that is produced by many human cancers [18, 19]. Epiregulin stimulated keratinocyte proliferation and phosphorylation of ribosomal protein S6 in vitro. Our results suggest that hamartomatous TSC skin tumors are induced by paracrine factors released by “two-hit” cells in the dermis, and that proliferation with mTOR activation of the overlying epidermis is an effect of epiregulin.

Development of xenograft mouse model for studies of TSC skin hamartomas

Xenograft models for several types of human cancer have been useful for both basic science studies and preclinical testing of novel candidate drugs [20]. To elucidate mechanisms of tumor formation and study drug action in vivo, there was a great need for a xenograft model of TSC skin hamartomas. Xenografted skin equivalents have been used by many investigators to study different aspect of wound healing and angiogenesis [21, 22]. Our goal was to develop an experimental model of TSC skin hamartomas by grafting constructs containing patient tumor-derived cells to mouse skin [23]. Fibroblast-like cells were grown from angiofibromas, periangual fibromas, or forehead plaques and normal-appearing skin from TSC patients. Cells from TSC patients were selected for use in the animal model based on the tumor-derived cells showing undetectable TSC2 protein. Normal fibroblasts or tumor cells from TSC patients were incorporated to mouse skin tumor cells. Rapamycin decreased numbers of TSC2-null tumor-derived cells showing in vivo. Our results demonstrated that TSC skin hamartoma cells promote tumor formation and study drug action in vivo, there was a great need for a xenograft model of TSC skin hamartomas.

Effects of rapamycin on TSC skin hamartomas

TSC tumors involve surgery, exposing patients to operative risks such as pain, functional deficits, recurrences, and scarring. The observation that tumor cells in TSC show loss of function of the TSC1-TSC2 complex and increased signaling through mTORC1 [6, 24] prompted clinical studies using mTOR inhibitors such as rapamycin (sirolimus) to treat TSC tumors. Sirolimus decreased the size of renal angiomylipomas, subependymal giant cell astrocytomas, lymphangioleiomyomas, chylous effusions and skin hamartomas [25]. It reduced the rate of decline of pulmonary function [26].

To study the potential utility and mechanism of action of rapamycin in the treatment of TSC skin hamartomas, we administered rapamycin to nude mice grafted with human TSC skin tumor cells. Rapamycin decreased numbers of TSC2-null cells and mononuclear phagocytes, and decreased angiogenesis and epidermal proliferation [23]. These results suggest that the decreased redness and size of TSC skin lesions observed in patients receiving systemic [27] or topical [28] rapamycin may result from both anti-tumor cell effects and anti-angiogenic effects. The antiangiogenic effects of rapamycin may be due to a direct inhibitory effect on vascular endothelium and/or indirect effects such as diminished release of angiogenic factors by TSC2-null cells or decreased recruitment of pro-angiogenic mononuclear cells.

SUMMARY AND CONCLUSIONS

TSC is an extremely complicated disorder, affecting different organ systems at different times in a patient’s life. In the past few decades, research studies of TSC disease mainly focused on genetic alteration of TSC genes and abnormalities in the PI3K/AKT/mTOR pathway. Those studies led to an effective drug treatment with rapamycin or its derivatives, which significantly benefit TSC patients. Unfortunately, rapamycin did not eradicate tumor cells so even better treatments are needed. We have demonstrated that TSC skin hamartoma cells promote tumor formation through interaction with surrounding cells directly or indirectly by releasing paracrine factors. These paracrine factors represent promising new targets for therapy, either alone or in combination with mTOR inhibitors. Future studies are expected to demonstrate the potential benefits of these new treatment strategies for curing TSC disease.

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