

REVERSION OF THE PHENOTYPE OF HUMAN $TSC2^{-/-}$ SMOOTH MUSCLE CELLS. NOVEL INSIGHTS FOR TSC AND LAM THERAPY

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Tuberous sclerosis complex (TSC) is a tumor suppressor gene syndrome resulting from the loss of function of the hamartin/tuberin complex. Two genes are implicated in TSC, *TSC1* and *TSC2*, respectively encoding hamartin and tuberin. The complex inhibits target of rapamycin (TOR)-mediated signaling to S6 kinase and participates in the control of cellular growth. From a surgically removed angiomyolipoma of a *TSC2* patient we have isolated alpha-actin-positive smooth muscle cells, named A^+ , bearing mutation on exon 18, with loss of heterozygosity for *TSC2* gene. S6K1 is constitutively activated, while IGF-1 is abundantly released and involved in cell survival rather than proliferation. EGF is necessary to promote proliferation of $TSC2^{-/-}$ A^+ cells. Antibodies to IGF-I or EGF receptors cause blockade in cell proliferation with total cell loss within 12 days. Following retroviral transduction of *TSC2* gene in A^+ cells, tuberin was successfully expressed and EGF-growth dependency was lost, thus it appears that EGF requirement for A^+ cell growth is due to lack of tuberin. $TSC2^{-/-}$ A^+ cells were positively labeled by HMB45 antibody, a marker for TSC and LAM cells, but following *TSC2* transduction, A^+ cells were negative. In *TSC2*-transfected cells phosphorylation of Akt and S6 was reduced, with the expression of Akt and S6 unaltered. Also PTEN phosphorylation decreased drastically. Exposure for 2 hours with IGF-1 (50ng/ml) slightly activated PI3K in $TSC2^{-/-}$ A^+ cells; this was not affected by inhibitors such as LY294002 (LY) and wortmannin. After reintroduction of *TSC2* gene the sensitivity to these inhibitors was restored and LY (20 μ M) addition reduced basal and IGF-1-induced Akt phosphorylation, and inhibited activation of S6K1 and PTEN. The phenotype of $TSC2^{-/-}$ A^+ cells was also assessed after antiEGFR and rapamycin incubation. When A^+ cells were exposed at plating time to antiEGFR (5 μ g/ml) labelling with HMB45 was down-regulated within 48 hours. A smaller effect was observed with rapamycin (5ng/ml). The proliferation rate of $TSC2^{-/-}$ A^+ cell was reduced by rapamycin when added at plating time, but a delay of 3 hours caused the loss of rapamycin effect. AntiEGFR was effective when added in both conditions. In conclusion our study shows that lack of tuberin affects greatly the EGF dependency for growth and IGF-1 related metabolism. The insensitivity to IGF-1 pathway inhibitors is reversed by the reintroduction of *TSC2* gene. The superior effects of antiEGFR versus rapamycin suggest a novel therapeutic strategy for TSC and LAM.