

## **PROLIFERATION AND INVASIVENESS OF HUMAN SMOOTH MUSCLE TSC2<sup>-/-</sup> CELLS. EFFECT OF ANTI-EGFR AND RAPAMYCIN**

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Tuberous sclerosis complex (TSC) is an autosomal dominant syndrome with variable penetrance characterized by neurologic, renal, and dermatologic manifestations. The tumor suppressor genes *TSC1* and *TSC2* encode hamartin and tuberin, respectively, and their loss of function can be associated with lymphangiomyomatosis (LAM). LAM is a lung disorder characterized by abnormal growth of smooth muscle cells which leads to cystic destruction of lung and loss of pulmonary function. Cultured human TSC2<sup>-/-</sup> cells isolated can be a source of useful informations for developing more appropriate pharmacological strategies aimed at blocking the life-threatening growth of smooth muscle cells in TSC and LAM. We have recently reported the isolation of human TSC2<sup>-/-</sup> smooth muscle cells, named A<sup>+</sup> cells, from a renal angiomyolipoma characterized by EGF-dependent proliferation, hyperphosphorylation of S6K and S6 and positivity to HMB45 antibody. Exposure to antibodies to EGF and IGF-1 receptors causes the progressive loss of A<sup>+</sup> cells. To evaluate *in vivo* such a therapeutic potential we created a model of TSC complications and LAM, by applying TSC2<sup>-/-</sup> cells to mice. 5 weeks old Hsd:Athimic Nude-nu nu/nu mice were *i.p.* injected with 5x10<sup>5</sup> A<sup>+</sup> cells previously labeled with PKH26-GL. Lungs, kidneys, uteri and cervical, mediastinic and retroperitoneal lymph nodes were dissected out and properly processed after 30 days, 90 and 180 days. Labelled TSC2<sup>-/-</sup> cells were detected. A diffuse positivity to HMB45 antibody and a strong S6 phosphorylation were observed in lymph nodes and uteri and in to lesser extent in lungs that presented an emphysema picture with cystic degeneration. All the controls injected with normal human aorta smooth muscle cells did not present lesions. Recently we developed the nasal administration of TSC2<sup>-/-</sup> cells, in such a way the respiratory system is quickly invaded. 2x10<sup>5</sup> A<sup>+</sup> cells were applied and in alveolar lung walls and lymph nodes were quickly and massively infiltrated, especially the mediastinic lymph nodes. Invasive A<sup>+</sup> cells were HMB45 positive and expressed S6 constitutive phosphorylation. 30 and 60 days after nasal administration a strong progressive destruction of lung parenchyma was observed. The administration of anti-EGFR caused a drastic reduction in the number of TSC2<sup>-/-</sup> A<sup>+</sup> cells present in lungs and reduced the structural alterations. Rapamycin was poorly effective. The killing activity of anti-EGF-R antibodies *in vitro* and *in vivo* suggest a new therapeutic approach capable of controlling the abnormal smooth muscle cell growth in TSC complications and LAM.