Human LAM/TSC cells efficiently cause lung nodule formation and alveolar destruction. Pharmacological counteraction by modulation of mTOR activity.

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Lymphangioleiomyomatosis (LAM) is a rare, low-grade neoplasm affecting almost exclusively women of childbearing age (McCormack et al., 2012). It occurs sporadically or in association with Tuberous Sclerosis Complex (LAM/TSC) a genetic, multisystem disease. LAM is characterized by cystic remodeling of the lung parenchyma and extra-pulmonary manifestations involving lymphatic channels due to proliferation of abnormal smooth muscle-like cells defined LAM cells. In lung tissue LAM cells caused destruction of parenchyma, obstruction of lymphatics, formation of nodules and cysts, leading to spontaneous pneumothoraces and progressive loss of pulmonary function.

In our laboratory, α-actin smooth muscle cells have been isolated from chylous effusion of a LAM/TSC patient (LAM/TSC cells). These circulating cells showed reactivity to HMB45 and CD44v6 antibodies, markers of TSC and LAM. Proliferation of these cells depends on epidermal growth factor (EGF) and antibodies directed to EGF receptor (anti-EGFR Ab) gradually reduced proliferation and caused cell death. We previously developed a mouse model using $TSC2^{-/-}$ ASM cells isolated from an angiomyolipoma (Lesma et al., 2012). We think that the different origin of LAM/TSC cells, their ability to survive in nonadherent condition and their mesenchymal features may allow to develop a new animal model with complementary characteristics to the previous mouse model to better study the pathogenesis of LAM.

We studied the histopathological changes in lungs after 3, 6 and 12 months from LAM/TSC cells administration. LAM/TSC mice showed progressive alveolar destruction. LAM/TSC cells infiltrated into pulmonary alveolar walls and caused airspaces enlargement as we previously demonstrated after $TSC2^{-/-}$ ASM cells administration (Lesma, et al.; 2012). In LAM, pathological changes are characterized by the development of pulmonary cysts and by nodules. Differently from $TSC2^{-/-}$ ASM cells, LAM/TSC cells administration was associated to the development of nodules in lung parenchyma 6 and 12 months after cells administration in a time dependent manner. 77% and 85,7% of mice developed lung nodules 6 and 12 months from cells inhalation, respectively. Localization tended to be near lymphatic and blood vessels or airways, such as in human LAM. Within the nodules we identified cells of human origin for their positivity to human COX IV and HLA-ABC antibodies. Moreover, cells positive to α-actin smooth muscle antibody, marker of LAM cells, were detected. In the nodules several cells expressing phosphorylated S6, the molecular signature of mTOR activation in LAM, were observed. Hormones play a central role in LAM pathogenesis. Estrogen enhances the neoplastic potential and survival of LAM cells and it has been demonstrated that estrogen and progesterone receptors are expressed in lung of LAM patients (Matsui et al, 2000). Likewise, we identified positivity of estrogen and progesterone receptors in lung nodules of mice.

Treatments with anti-EGFR Ab and rapamycin decreased the percentage of LAM/TSC mice with nodules to 33% ($p<0,05$ *vs* LAM/TSC mice) and 50% (n.s.), respectively. Moreover, pharmacological treatments significantly ameliorated histopathology of lung tissue reducing alveolar destruction. Rapamycin appear to be less effective than anti-EGFR Ab and caused haemoptysis, as we previously observed in the model obtained with $TSC2^{-/-}$ ASM cells.

These data demonstrate that inhalation of LAM/TSC cells in nude mice allowed to develop a LAM model with lung nodules, differently from the model developed with $TSC2^{-/-}$ ASM cells, and caused alveolar destruction. Administration of LAM/TSC cells seems to better reproduce the histopathological features of LAM. Rapamycin and, more significantly, anti-EGFR antibody reduced pulmonary metastases and lung damage.

Lesma, et al. *Am J Pathol.* 181(3), 947-960
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