Direct and indirect pharmacological regulation of mTOR affects fate of LAM/TSC cells. Epithelial to mesenchymal transition features of LAM/TSC cells

S. Ancona[1], E. Lesma[1], S.M. Sirchia[1], E. Orpianesi[1], E. Chiaramonte[1], A.M. Di Giulio[1], A. Gorio[1]

Dept. of Health Sciences, Università degli Studi di Milano, Milano, Italy

Lymphangioleiomyomatosis (LAM), a rare lung disease leading to progressive respiratory failure, is characterized by widespread pulmonary proliferation of abnormal smooth muscle-like (ASM) cells leading to destruction of lung parenchyma, fluid-filled cystic structures in axial lymphatics, and abdominal tumors. LAM cells underlie the formation of characteristic LAM nodules responsible for cystic destruction and angiomyolipomas (AMLs) in kidneys (Johnson, 2006) . LAM can be sporadic or associated with tuberous sclerosis complex (TSC) (Carsillo et al., 2000). TSC is an autosomal dominant syndrome characterized by hamartoma-like tumor growths in various organs such as brain, kidney, skin, retina, and heart (Curatolo et al., 2008). TSC is caused by mutations in *TSC1* or *TSC2* genes, encoding hamartin and tuberin, respectively. Identical *TSC2* mutations and loss of heterozigosity (LOH) patterns were found in LAM cells from lung nodules, AMLs, and lymph nodes of the same sporadic LAM patient suggesting that the two diseases share a common genetic origin; this is consistent with metastatic spread among organs (Karbowniczek et al., 2008). The absence of tuberin in smooth muscle-like cells from AML of a TSC2 patient caused by methylation of the *TSC2* promoter was recently described (Lesma et al., 2009). Such behaviour cells with respect to their infiltrative growth pattern, metastatic potential, and altered cell differentiation is reminiscent of cells undergoing epithelial to mesenchymal transition (EMT) (Hugo et al., 2007). From chylous of a patient affected by LAM associated to TSC, α-smooth muscle like cells were isolated and characterized. These cells were positive to HMB45 and CD44v6 antibodies and bear a germline *TSC2* mutation in exon 21. An epigenetic alteration as second hit was demonstrated by tuberin expression after exposure to chromatin-remodeling agents and the presence of a large amount of non-coding DNA with closed chromatin regions. LAM/TSC cells undergo spontaneous cycles of adhesion and nonadhesion conditions, likely mediated by the inactivation of the focal adhesion kinase (FAK)/Akt/mTOR pathway, and display the ability to survive independently from adhesion. In LAM/TSC cells FAK inhibition caused the reduction of Akt phosphorylation which was followed by inhibition of mTOR phosphorylation and mTOR autophosphorylation and consequently by a strong reduction of S6 phosphorylation as it occurs in nonadherent cells. In adherent cells S6 and Erk phosphorylation were much higher than in nonadherent cells. Moreover, nonadherent LAM/TSC cells underwent an extremely low rate of proliferation consistent with the tumour stem cell characteristics. One of the step driving EMT is the repression of E-cadherin, resulting in the loss of cell-cell adhesion. Adherent and nonadherent LAM/TSC cells did not express E-cadherin, but both expressed vimentin, marker of mesenchimal cells. Anti-EGFR antibodies and rapamycin affected proliferation and viability of nonadherent cells. EMT process controls the migration of cancer cells from primary tumors depending on an inflammatory microenvironment. LAM/TSC cells secreted high amount of interleukin(IL)-6 and IL-8, cytokines with a crucial functional role in a variety of cancer cells (Condeelis et al., 2003). In conclusion, the understanding of LAM/TSC cell features may result important in the assessment of pathological cell invasiveness in LAM and TSC, and should provide a useful model to test therapeutic approaches aimed at controlling their migratory ability.

Carsillo et al., (2000). *Proc Natl Acad Sci USA.* 97, 6085-6090.
Curatolo et al., (2008). *The Lancet.* 372, 657-668.
Johnson, (2006). *Eur Respir J.* 27, 1056-1065.
Karbowniczek et al., (2003). *Am J Respir Crit Care Med.* 167, 976–982.
Lesma et al., (2009). *Am J Pathol.* 174, 2150-2159.
Hugo et al., (2007). *J Cell Physiol.* 213, 374–383.
Condeelis et al., (2003). *Nat Rev Cancer.* 3, 921-30.