Matrix metalloproteinases-7 and -2 contribute to the development of LAM lesions. Sensitive regulation by EGF receptor

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

[1]Laboratory of Pharmacology, Dept. of Health Sciences, Università degli Studi di Milano, Milano, Italy

Matrix metalloproteinases (MMPs), a group of zinc-dependent endopeptidases, are a family of enzymes involved in many processes such as extracellular matrix remodelling, angiogenesis, migration and cell signalling. Imbalance between MMPs and their inhibitors is implicated in a variety of pulmonary disorders including lymphangioleiomyomatosis (LAM) (Chang et al., 2010). LAM is a progressive interstitial lung disease leading to loss of pulmonary function, characterized by destruction of lung parenchyma with deposits of spindle-shaped and epithelioid cells termed LAM nodules. LAM is associated with the functional inactivation of the tuberous sclerosis complex 1 (*TSC1*) and *TSC2* genes. Loss of function of tuberin, the product of *TSC2* and a negative regulator of the mammalian target of rapamycin (mTOR) signaling pathway, results in the phenotypic manifestation of TSC. Pulmonary lesions, serum and urine from LAM patients contain increased levels MMP-2 and MMP-9. Although many MMPs are produced by the stromal cells surrounding cancer cells, MMP-7 is produced by the cancer cells itself and it has been shown to be overexpressed in several types of invasive cancers. MMP-7 has a powerful proteolytic activity and broad substrate specificity with an important role in invasion and metastasis of several carcinomas including lung carcinomas. In this study, we characterized for the first time MMP-7 and MMP-2 expression in human LAM/TSC cells and lungs from LAM and LAM/TSC patients, and we evaluated the effect of pharmacological treatments with anti-EGFR antibody and rapamycin in an *in vivo* LAM model.

MMP-7 and MMP-2 were highly expressed in lungs of LAM and LAM/TSC patients. MMP-7 was widely diffused as it occurs for MMP-2. This data suggest that MMP-7 as MMP-2 may contribute to ECM degradation and development of LAM lesions, leading to the respiratory dysfunction. We recently isolated and characterized LAM/TSC cells from chylous effusion (Lesma et al., 2014). These cells do not express tuberin for an epigenetic modification, bear mesenchymal features and survive in adherent and nonadherent condition. MMP-7 mRNA levels were increased in cells growing independently from anchorage while MMP-2 mRNA levels were higher in adherent cells. Overall, MMP-7 and MMP-2 mRNA expression was decreased in LAM/TSC cells expressing tuberin after 5-azacytidine treatment, a chromatin remodelling agent. CD147 extracellular metalloproteinase inducer (EMMPRIN), described as an inducer of the expression of MMPs and recently appreciated to have multiple roles including migration, adhesion, invasion, energy metabolism, was significantly reduced by 5-azacytidine exposure. These data confirm a modulation of MMPs by tuberin expression and, likely, hamartin-tuberin complex function.

In lungs of an *in vivo* LAM model developed by endonasal administration of LAM/TSC cells in nude mice, the expression of MMP-7 and MMP-2 was much higher than in control mice. MMP-7 and MMP-2 were mainly localized in lung nodules. We have described that LAM/TSC cell proliferation is dependent from EGF and blocking the receptor with anti-EGFR antibody causes cell death. Following treatment with anti-EGFR antibody the expression of MMP-7 and MMP-2 was significantly decreased. Rapamycin, an mTOR inhibitor, had a lower effect on MMP-7 expression.

These data indicate that MMP-7 as well as MMP-2 may contribute to ECM degradation and development of cystic lesions, an important aspect of LAM pathology that likely contributes significantly to respiratory dysfunction. Anti-EGFR antibody was more efficient than rapamycin in reducing MMP-7 expression in lung nodules caused by LAM/TSC cells in nude mice suggesting that the the inhibition of EGFR signalling has a potential in treatment of LAM/TSC lung alterations.

Chang WY, et al., Am J Physiol Lung Cell Mol Physiol. 2010;299(3):L393-400.
Glasgow CG, et al., Respir Med. 2010;104 Suppl 1:S45-58
Lesma E, et al., J Cell Mol Med. 2014;18(5):766-79