Matrix metalloproteinase 2 and 7 are new targets in LAM and TSC-related disorders

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Tuberous sclerosis complex (TSC), an autosomal dominant disease characterized by the formation of hamartomas in various organs, is caused by mutations in *TSC1* e *TSC2* tumor suppressor genes, encoding hamartin and tuberin, respectively. Lymphangioleiomyomatosis (LAM) is a rare lung disease characterized by cystic lung destruction and resulting from proliferation of neoplastic cells bearing mutations in either *TSC1* or *TSC2* genes. A metastatic process has been proposed in dissemination of LAM and TSC cells to explain the ability to cause hamartomas in various organs. Matrix metalloproteinases (MMPs) degrade and modify the extracellular matrix (ECM), facilitating detachment of cells from the tissue. Levels of MMP-2 and MMP-9 in urine are predictive of disease status in a variety of cancers and also in LAM. MMP-2 and MMP-9 levels are high in urine of LAM patients and MMP-2 is substantially up-regulated in their lung tissue (Glasgow et al.,2010). Matrilisin (MMP-7) contributes to tumor progression, invasion and is overexpressed in several types of invasive cancers (Barnes et al., 2010).

The aim of this project is to study the role of MMP-2 and MMP-7 in motility in human cells isolated from a LAM/TSC patient. LAM/TSC cells, isolated from chylous of a patient affected by LAM associated to TSC, bear a *TSC2* germline mutation and do not express tuberin for an epigenetic modification. These cells, such other cells previously isolated in our laboratory, required epidermal growth factor (EGF) to proliferate and the blockade of EGF receptor causes cell death (Lesma et al., 2005; 2009). LAM/TSC cells survive in adherent and nonadherent condition and bear mesenchymal characteristics. For these features these cells are a good model to study the mechanisms of motility in LAM and TSC and the relation to tuberin expression. We studied the effect of anti-EGFR Ab and rapamycin on motility and MMPs expression.

In adherent status LAM/TSC cells expressed higher levels of MMP-2 mRna and lower of MMP-7 than in nonadherent condition, consistent with invasive features. MMP-2 and MMP-7 mRna expression appeared to be related to tuberin since they were significantly reduced by 5-azacytidine. Anti-EGFR Ab and rapamycin significantly decreased MMP-2 mRna expression while their effect was the opposite on MMP-7.

Extracellular matrix metalloproteinase inducer (EMMPRIN), is thought to affect tumor progression through its ability to stimulate MMP expression (Odajima et al., 2010). EMMPRIN expression, quantified by cytometric analysis, was reduced by 5-azacytidine and was much higher in LAM/TSC cells than in MCF7 cells. Motility and MMPs expression were evaluated in wound healing assay providing an indication of cell migration rate. LAM/TSC cells close wounds in 11h, much faster than MCF7 cells. Following 5-azacytidine treatment, and the consequent induced tuberin expression, LAM/TSC cells reduced their motility and MMP-2 mRna was significantly increased. Also rapamycin, an mTOR inhibitor, and anti-EGFR Ab decreased the migration rate but at a slightly lower extent than 5-azacytidine, leading to the closure of wound in about 29h. In wound healing condition rapamycin and anti-EGFR Ab induced an increase of the levels of MMP-2 even higher than 5-azacytidine treatment.

In conclusion our data suggest a role for MMP-2 and MMP-7 in LAM/TSC cell mobilization and thereby in the pathogenesis of LAM and TSC indicating their involvement in the metastatic process proposed in dissemination of LAM cells. The MMPs differences between LAM/TSC in adherent and in nonadherent status highlight the potential for targeting the MMP-7 pathways as novel therapeutic approaches in LAM and other TSC-related disorders.

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