EGF contributes to the pathogenesis of lymphangioleiomyomatosis by affecting ERK pathway

S. Ancona[1], A. Prinelli[1], E. Chiaramonte[1], A.M. Di Giulio[1], A. Gorio[1], C. Pesenti[2], A. Baisi[3], S.M. Sirchia[4], E. Lesma[1]

[1]Laboratory of Pharmacology, Dept. of Health Sciences, Università degli Studi di Milano, Milano, Italy
[2]Division of Pathology, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milano, Italy
[3]Thoracic Surgery, Dept. of Health Sciences, Università degli Studi di Milano, Milano, Italy
[4]Medical Genetics, 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 cells. LAM cells underlie the formation of characteristic LAM nodules responsible for cystic destruction of lung parenchyma and kidneys angiomyolipomas (AMLs). LAM lesions are heterogenous structures formed by cells of different phenotypes that permit the environmental events for the development of the disease. LAM can be sporadic or associated with tuberous sclerosis complex (TSC) (Carsillo et al., 2000). TSC is caused by mutations in *TSC1* or *TSC2* genes, encoding hamartin and tuberin, respectively. Tuberin and hamartin form a complex (TSC complex) that regulate the mammalian target of rapamycin (mTOR) and then cell growth. mTOR is present within two protein complexes in the cell: mTORC1, bound to raptor, and mTORC2, coupled to rictor. mTOR is involved in signal transduction activated by epidermal growth factor (EGF) receptor (EGFR). EGFR activation initiates multiple pathways involving effector such as PI3K/AKT and MAPK/ERK. Moreover, the aberrant expression of EGFR has an important role in the development and growth of tumor cells. We previously demonstrated that EGF is necessary for TSC2-null cell proliferation.

To study the role of EGFR in the pathogenesis of LAM and TSC, we analyzed the expression of EGFR in LAM/TSC lung patients. Moreover, we isolated cells from lung patients to study the cascades activated by EGF and to evaluate the role of EGFR as a therapeutic target for the treatment of LAM and TSC. Isolated lung cells were positive to CD44v6, marker of LAM cells (Pacheco-Rodriguez et al., 2007). We characterized these cells as positive or negative for HMB45 antibody (lung cells^{HMB45+} and lung cells^{HMB45-}), pathological marker of TSC cells (El-Hashemite et al, 2005). Lung cells bear mutations in *TSC2* gene without LOH and expressed tuberin even if a lower extent than HASMCs, used as control. Lung cells^{HMB45+} have slightly lower levels of tuberin than HMB45-negative population. It is reasonable to speculate that the impaired tuberin expression caused deregulation of TSC complex. Proliferation of lung cells^{HMB45+} and cells $^{HMB45-}$ was EGF-dependent and the exposure to the antibody directed against EGFR inhibited cellular proliferation, as we previously demonstrated for TSC2-null cells (Lesma et al., 2014). In lung cells, EGFR was expressed at high levels and the analysis for the more common EGFR mutations, usually observed in lung cancer, did not show any alterations. Consistent with this data, in lungs of LAM/TSC patients, EGFR was highly expressed. In both lung cells $^{HMB45+}$ and cells^{HMB45-}, EGFR was dependent on the EGF availability. These data suggest a role of EGFR in the development of LAM lesions. In lung cells, EGF withdrawal caused an increase of ERK phosphorylation while in HASMCs the absence of EGF did not alter ERK activation. Akt and S6 phosphorylation were reduced when EGF was removed from the medium. Akt was more quickly activated by EGF in lung cells than in HASMCs. Anti-EGFR antibody did not have any effect on ERK phosphorylation after one hour incubation, while a slightly reduction was observed in 24 hours.

In conclusion, in lungs of LAM patients heterogeneous populations for pathological phenotype, HMB45 positive or negative cells, share the dependency from EGF to proliferate and the modulation of targets of EGFR pathway such as the specific sensitivity of ERK activation. In this perspective, therapeutic approaches for LAM and TSC, such as anti-EGFR antibodies, need to be explored.

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