of morbidity and mortality due to chronic infections and unresolved inflammation. The reduction in the airway surface liquid (ASL) pH is one of the hypotheses that tried to explain the high susceptibility to lung infections. Together with a reduced bicarbonate transport through CFTR, an increase in lactic acid secretion could also explain the changes in extracellular pH. The aim of the present work was to determine if the EGFR pathway is involved in the pH regulation in CF cells. Two cellular models were used: IB3-1 cells (bronchial epithelial cells derived from a CF patient with a ΔF508/W1282X CFTR genotype) and C38 cells (IB3-1 "corrected" cells). The results reported a decrease in pH in the extracellular medium culture ($p<0.05$) in IB3-1 cells concomitantly with an increased in lactate secretion and both LDH expression and activity ($p<0.05$) compared with C38 cells. These results confirmed that the CFTR regulates significantly ($p<0.05$) the pH, lactate secretion and LDH expression and activity. As we have previously observed the role of the EGFR pathway in the CF phenotype, we studied if this signaling pathway is also involved in pH regulation. Here, we observed that EGFR modulates significantly ($p<0.05$) the pH in the extracellular medium, the lactic acid (lactate) secretion ($p<0.05$) and the LDH expression and activity ($p<0.05$) in CF cells. In conclusion, our results showed that CFTR channel activity (or expression) regulates not only the pH, but also the lactic acid secretion and LDH expression and activity and the EGFR pathway is partially involved in this regulation. The low pH microenvironment observed in CF cells could promote the impairment of immune function and consequently the establishment of infections in people with CF (PWCF). Acknowledgements: ANPCYT, UCA and CONICET.

356. (352) **INTERCELLULAR MITOCHONDRIAL TRANSFER THROUGH NANOTUBULES IS PROMOTED BY CYCLIC AMP (cAMP) IN RAT ASTROCYTES AND HUMAN GLIOBLASTOMA CELLS**

Helfenberger K[1], Benzo Y[1], Duarte A[2], Fuentes F[2], Poderoso JJ[3], Maloberti P[1], Poderoso C[1].
1. Departamento de Bioquímica. Facultad de Medicina. Instituto de Investigaciones Biomédicas (INBIOMED, UBA-CONICET). Universidad de Buenos Aires.
2. Academia Nacional de Medicina. Instituto de Medicina Experimental (IMEX-CONICET).
3. Hospital de Clínicas "José de San Martín". Facultad de Medicina. Instituto de Inmunología, Genética y Metabolismo (INIGEM, UBA-CONICET). Universidad de Buenos Aires.

Nanotubules (Tunneling nanotubules, TnTs) are cell membrane projections made of F-actin fibers of nanometric diameter (up to 1 μm), which enable cytoplasmatic connections between cells. It has been shown that mitochondria, other organelles, and cellular components are transferred by TnTs in several normal and tumor cell lines. TnTs establishment has been extensively described in nervous system cells, as neurons and astrocytes. Published evidence indicates the existence of mitochondrial transfer through TnTs between different cell types, such as normal and tumoral cells. Mitochondrial passage from normal to tumor cells restores oxidative metabolism, decreasing tumorigenic potential. A similar effect has been observed with cAMP, a very well-known astrocytes stellation promoter, which mediates mitochondrial biogenesis and tumor growth inhibition. Mitochondrial transfer within TnTs in nervous system cells have not been demonstrated so far. Then, our goal was to analyze mitochondrial trafficking through TnTs in normal and tumoral astrocytes and a possible effect of cAMP. We used normal rat astrocytes and human glioblastoma U87 cells. Mitochondria and actin were probed with a mito-targeted green fluorescent protein and phalloidin, respectively. Astrocytes and U87 were incubated with or without 8Br-cAMP (cAMP analogue). We analyzed images by confocal microscopy and measured the width of actin connections between cells. We analyzed each culture separately; astrocytes and U87 establish thick projections containing mitochondria but treatment with cAMP promotes an increase of TnTs-like connections with mitochondria inside (control vs cAMP: astrocytes: 2.07±0.71 vs 0.85±0.21 μm, ***$p<0.05$; U87: 2.69±1.40 vs.0.84±0.22 μm, *$p<0.05$, ± SD, ANOVA, Tukey test). Thus, cAMP promotes TnTs-like structures and mitochondrial passage through them in normal astrocytes and glioblastoma cells, suggesting a role for intercellular mitochondrial transfer through TnTs in stellation process.

357. (416) **TPR-PROTEINS INFLUENCE THE NUCLEOCYTOPLASMIC SHUTTLING OF GR**

Mazaira GI[1], Ciucci SM[1], Echeverría P[2], Galigniana MD[1,3]
1. Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires.
2. Département deBiologie Cellulaire, Université de Genève
3. Instituto de Biologiá y Medicina Experimental (IBY-ME)-CONICET.

Glucocorticoid receptor (GR) exists as a heterocomplex with the chaperone Hsp90 and a co-chaperone carrying a tetratricopeptide-repeat (TPR) domain through which it interacts with Hsp90. Steroid binding promotes the exchange of TPR proteins on the GR-Hsp90 complex, such that FKBP51 is replaced by its homolog partner FKBP52, an immunophilin that interacts with dynein motors favouring the retrograde transport of GR. In this study, we hypothesized that TPR-domain proteins regulate the subcellular localization of GR. It is shown that GR nuclear accumulation is impaired by overexpression of the recombinant TPR peptide. This disrupts the association between GR-Hsp90 an FKBP52, and consequently, the association with dynein is lost. It also causes loss of association with the importin-β1 adapter transporter (KPNB1), the nuclear pore-associated glycoprotein Nup62 and structures associated with the nuclear matrix. The final GR distribution is the result of the combination of two processes: decreased nuclear import and improved nuclear export. Interestingly, leptomycin B (a CRM1/exportin-1 inhibitor) abolished the effects of TPR peptide overexpression despite not having inhibitory effect itself on the nuclear GR export. These results strongly suggest the existence of a TPR domain-dependent mechanism for nuclear protein export. In summary, our study demonstrates a strong relationship between TPR proteins and the nuclear import mechanism of the receptor, as well as their potential capability to favour the anchorage of the GR to nuclear structures. We propose that the balance of expression of the TPR domain proteins bound to the GR-Hsp90 complex can determine the subcellular localization and the nucleocytoplasmic properties of the receptor and, therefore, its pleiotropic biological properties in different tissues or cell types.
KEY WORDS: Glucocorticoid receptor, Tetratricopeptide repeats, Immunophilins, FKBP52, Hsp90, Nuclear matrix

358. (527) **ACTIVATION OF THE LIVER X RECEPTOR (LXR) INHIBITS INFLAMMATORY EFFECTS ASSOCIATED WITH INVOLUTION AND PROMOTES LACTOGENIC FEATURES IN MOUSE MAMMARY CELLS**

Bogni ES[1], Grinman DY[2], Vallone S[1], Gatelli A[1], Pecci A[1], Meiss R[3], Kordon EC[1].
1. Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE), Facultad de Ciencias Exactas y Naturales (UBA).
2. Department of Internal Medicine, School of Medicine, Yale University (USA).
3. División de Patología Experimental, Academia Nacional de Medicina (ANM).

It is well-known that lactation is driven by glucocorticoids and prolactin (Prl) that induce milk protein expression through STAT5 activation. On the other hand, post-lactational involution is characterized by the secretion of pro-inflammatory factors that trigger mammary cell apoptosis. LXR is an anti-inflammatory transcription factor present in all developmental stages of the mouse mammary gland.The goal of this study was to determine whether LXR plays a relevant role during the lactation/involution switch. Female C57/bl6 mice were treated with the LXR agonist GW3965 (10 mg/kg) or DMSO (control) by IP injection at weaning, 48h or 96h post weaning (n=3 per group). At those times, mice were euthanized and pieces of their inguinal mammary glands were either fixed in formalin or frozen at -800C. Fixed tissue was later processed for histological studies, while proteins and RNA were extracted from frozen samples. On the other hand, we analyzed the effects of GW3965 (10-6M) compared to Dexamethasone (Dex), Dex+Prl or DMSO for 72h on HC11 mouse mammary cells in culture. Our results show that LXR activa-