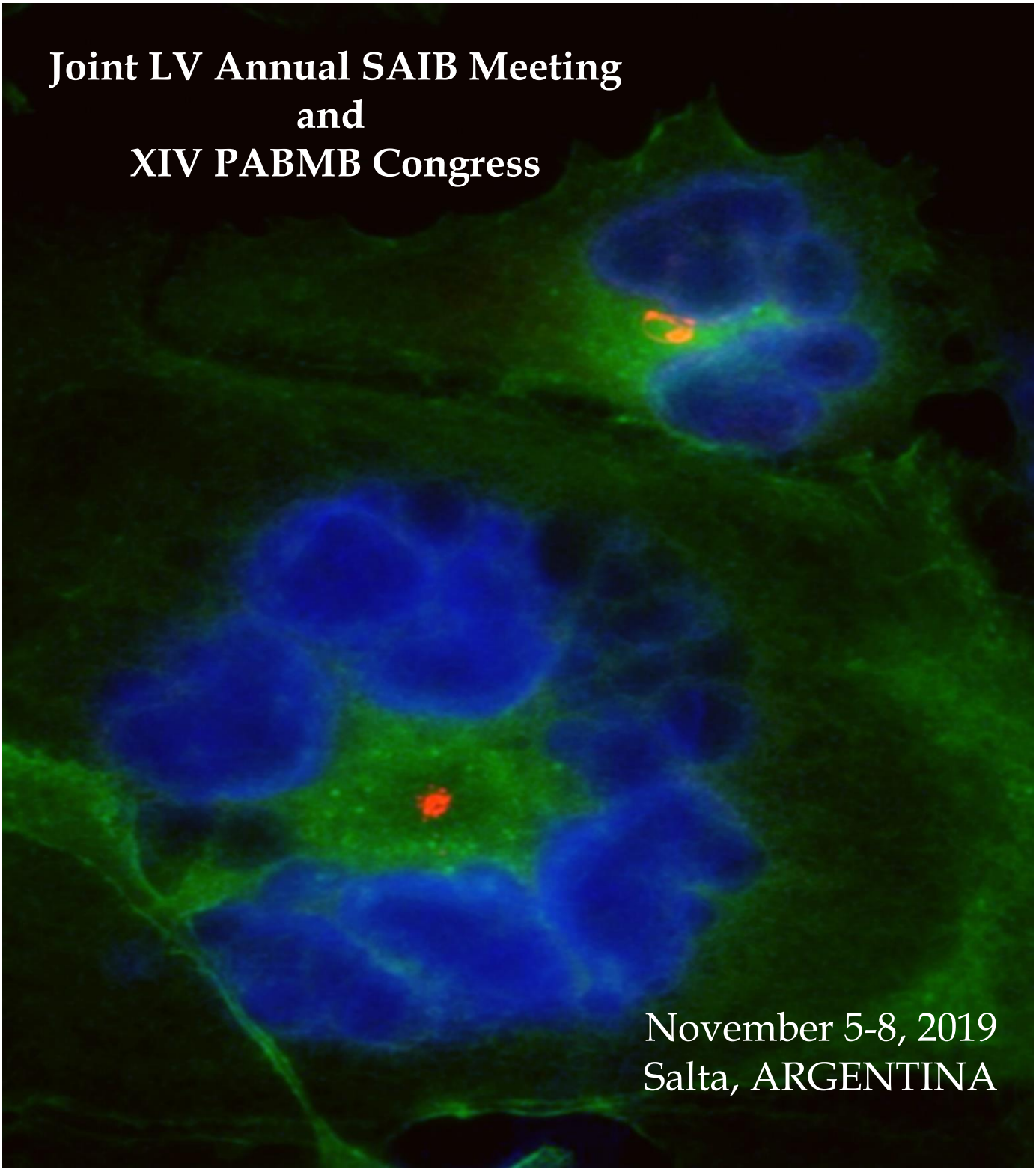

Joint LV Annual SAIB Meeting and XIV PABMB Congress

November 5-8, 2019
Salta, ARGENTINA

important roles in late long-term potentiation and long-term memory. Additionally, PKCzeta/ PKMzeta aggregates are present in the neurofibrillary tangles that affect brain function of Alzheimer's disease patients. We have previously described the mechanisms used by PDK1 to sense the conformation of its substrates during cell signaling. We now show through a variety of techniques that blocking the "conformational sensor" pocket on PDK1 can trigger the cellular aggregation of PKCzeta. Based on our results, we elaborated a novel hypothesis that can explain how proteins aggregate in conformational disorders and the existence of "strains". The main goal of the follow-up project is to identify specific conformational sensors that are responsible for the cellular aggregation of Tau, alpha-synuclein, and p62/SQSTM1, all proteins that aggregate in conformational disorders. We envisage that the identification of the "conformational sensors" and the actual mechanisms involved in protein aggregation will enable development of innovative drugs that target the original cause of major global diseases that have highly unmet medical needs.

CB-P18

PROTEOLYTIC FRAGMENTS OF GHRELIN N-TERMINUS SHOW DIFFERENTIAL OREXIGENIC EFFECTS THAN THE PARENTAL HORMONE

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The stomach-derived hormone ghrelin was first reported as a growth hormone secretagogue and a "hunger" hormone that stimulates food intake and the preference for energy-dense foods. However since its discovery in 1999, the number and kind of activities reported for ghrelin has been increasing over time, including gut motility and gastric secretion/emptying, regulation of adiposity, blood glucose levels, bone metabolism, sleep, stress and hedonic feeding, enhancement of contractility and vasodilatation. Ghrelin is a peptide of 28 residues acylated with an octanoic acid at Ser3. The N-terminal sequence of ghrelin along with the octanoyl group are essential to act on the ghrelin receptor (GHSR1a) through which the hormone triggers its effects. As many peptide hormones undergo proteolytic processing as a regulatory mechanism–producing fragments that may differ not only in the magnitude of the effects but also the type of bioactivity they exert–here, we explored the hypothesis that circulating ghrelin can be cleaved in order to generate ghrelin-derived peptides with differential bioactivities. Initially, by in vitro digestion of ghrelin with human plasma followed by MALDI-TOF MS detection, we found that the bonds of ghrelin sequence extended from residue 11 to 16, are hydrolyzed by proteases present in plasma. Then, we also incubated ghrelin with a human hepatocarcinoma cell line (HepG2) or with the extracellular medium of a 48h-culture of these cells. The MALDI-TOF MS analysis of these digests showed the same "hot cleavage zone" in ghrelin sequence previously found with plasma digestion samples. In addition, using a fluorescent analogue of ghrelin, extracellular medium as source of proteases and different proteases inhibitors, we have been able to elucidate that HepG2 cells secrete, at least, two different proteases able to cleave ghrelin peptide bonds. In order to evaluate the impact of proteolysis on the orexigenic effects of ghrelin, we tested one of the ghrelin-derived fragments detected in MALDI-TOF MS analysis of in vitro digests (ghrelin(1-14)). Despite having the active core of the hormone, we found that ghrelin(1-14) failed to induce neuronal activation, assessed by the marker of neuronal activation c-Fos, nor to increase food intake in mice. Additionally, these ghrelin-derived peptide was unable to impair the orexigenic effect of full-length ghrelin in competition assays. Together, these data support the existence of a proteolytic extracellular mechanism that generates ghrelin-derived peptides with different bioactivity than full-length ghrelin. Moreover, the liver may be involved in this mechanism during the passage of ghrelin through the hepatic portal circulation.

CB-P19

EXTRACELLULAR VESICLES SECRETION: INVOLVEMENT OF THE SNARE VAMP7 AND THE GTPASE Rab39a

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Secretion of extracellular vesicles (EVs) exerts fundamental roles in almost every tissue, in both physiological and pathological conditions. EVs emerge as important structures involved in cellular communication, particularly in cancer, autoimmunity, infectious diseases, and neurodegenerative disorders. These small membranous formations contain a vast amount of different molecules like nucleic acids, lipids, proteins, and sugars. Hence, EVs could be useful as novel biomarkers and therapies for the treatment of numerous pathologies. Understanding the role played by proteins involved in EVs exocytosis is crucial to control their burden. Rab proteins are the master controllers of vesicular transport. Our laboratory has demonstrated that Rab39a co-localizes with CD63 at multivesicular bodies (MVBs) and regulates the transport of these vesicles. We have shown the role of VAMP7 protein in EVs fusion with the plasma membrane. In this collaborative study, we found that Rab39a co-localized with VAMP7 in small vesicles. Furthermore, these proteins modulated EVs secretion in HeLa cells. Interestingly, in VAMP7-knockout cells, the amount of EVs released to the extracellular medium significantly decreased; therefore, EVs cargoes, including molecules with important functions in the immune system, were retained within cells. We found that Galectin-1, a protein of the lectin family, is released through VAMP7-dependent exocytosis. Consequently, impairing VAMP7 function might distort communication and signal transmission among cells, overall affecting immune response.

CB-P20

ANGIOTENSIN II, K AND CHEMOTHERAPEUTIC DRUGS INCREASE CELL RESISTANCE VIA ACSL4 AND ABCG2 IN ADRENOCORTICAL CARCINOMA

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