'LUCIO CHERNY FOUNDATION'- MULTIDISCIPLINARY

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0296 - INTEGRIN-MECHANOSIGNALING ROLE IN SMALL GTPASES ACTIVATION AND CANCER

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Abstract/Resumen: The ability of cells to adhere and simultaneously probe their mechanical environment is central to many physiological and pathological processes. Extracellular matrix sensing and mechanotransduction are mediated by the integrin family of cell adhesion receptors. Using genetically engineered cells, we studied the specific fibronectin integrin binding signaling and its role in tumor development. We observed that a5ß1-integrins promoted the formation of small adhesions, low RhoA activation and high force, while aß3-expressing cells showed large adhesions, thick stress fibers, high RhoA activation and low force. To further analyse these cellular phenotypes, we looked for specific RhoA activators (GEFs). For this purpose, we performed Mass Spectrometry (MS) analysis follow by biochemical assays and observed that GEF-H1 activation is aVß3-integrin dependent. Furthermore, using integrin-tail pull-down and MS assay, we observed that GEF-H1 binds to ß3-tail, suggesting that specific integrins may activate different Rho-GEFs during tumor progression. In order to study the role of GEF-H1 in cancer, we analysed by immunohistochemistry GEF-H1 expression in human biopsies. We observed overexpression of GEF-H1 in breast (p= 0.0053, n= 61) and thyroid (p= 0.0006, n= 32) tumor biopsies compared with normal tissue. Similar results were obtained in cancer cell lines (CCL). To further study the role of GEF-H1 in tumor development using CRISPR/Cas9 technology, we generated GEF-H1-knock out (KO) clones in a murine invasive breast CCL. We observed a decrease in the proliferation, migration and invasion rates ($p<0.001$) in GEF-H1-KO cells. These results showed that GEF-H1-RhoA activation is aVß3-integrin dependent and that may mediate the signaling involved in controlling cell structure, force generation, proliferation, migration and invasion of breast cancer cells. In addition, the studies in human tumor samples suggest that GEF-H1 might be a molecular biomarker in cancer.

0331 - REGULATORY MECHANISMS UNDERLYING FUNCTIONAL MATURATION OF SERTOLI CELLS IN RESPONSE TO ANDROGENS DURING POSTNATAL DEVELOPMENT

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Abstract/Resumen: Androgen-dependent maturation of Sertoli cells during postnatal testicular development is key for the establishment of spermatogenesis. Meiosis in the male begins at puberty and relies on androgens and retinoic acid. Immature Sertoli cells produce high levels of AMH, which is inhibited by androgens at puberty. The molecular mechanisms underlying androgen-mediated AMH decline are unknown. CYP26B1 degrades retinoic acid in the prenatal testis preventing meiosis initiation. The concurrence of meiotic entry and Sertoli cell maturation in response to androgens led us to propose that CYP26B1 —like AMH— is downregulated by androgens in the Sertoli cell during puberty, thus enabling meiosis initiation. By immunohistochemistry, we saw that CYP26B1 expression declines in the postnatal mouse Sertoli cell as androgen receptor (AR) expression increases, closely before meiotic spermatocytes appear. Luciferase reporter assays in the SMAT1 Sertoli cell line showed a direct negative effect on Amh promoter activity in the presence of dihydrotestosterone (DHT, $p<0.001$). Site-directed mutagenesis and ChIP-qPCR assays showed that androgen-mediated inhibition requires the SF1 sites in the Amh promoter. Regarding Cyp26b1, we saw no changes in promoter activity in response to androgens (p= 0.34). This lack of response was further supported by invariant levels of endogenous Cyp26b1 expression in SMAT1 cells transfected with the AR (p= 1.0) and in primary Sertoli cells of 10-day-old mice in culture (p= 0.7), after DHT treatment. ChIP-qPCR showed no enrichment in AR sequences analyzed, indicating a lack of functional binding of the AR. In sum, we confirmed a negative correlation between the immature Sertoli cell markers AMH and CYP26B1 and AR expression and meiotic initiation in postnatal development. We identified the molecular mechanism underlying AMH inhibition by androgens but found that the decline in CYP26B1 expression is not caused by a direct inhibitory androgen effect on Sertoli cells.

0359 - CDC42 ACTIVITY IS NECESSARY FOR THE INTERPLAY BETWEEN CAMP/PKA PATHWAY AND CATSPER FUNCTION

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Abstract/Resumen: Sperm acquire the ability to fertilize in the female genital tract in a process called capacitation. During capacitation, sperm undergo changes in the motility pattern called hyperactivation, which depends on Ca^{2+} transport by the sperm-specific Ca^{2+} channel CatSper. CatSper is essential for fertilization and therefore, it is subjected to a complex regulation that is not fully understood. Recent reports found that mouse CatSper is upregulated by cAMP-dependent activation of protein kinase A (PKA). From a molecular point of view, bicarbonate stimulation of the soluble adenylyl cyclase (sAC) leads to an increase in cAMP, PKA activity and tyrosine phosphorylation of sperm proteins. It remains incompletely understood if PKA itself phosphorylates CatSper or if its activation relays on other intermediary events. By using super-resolution microscopy, we report that similar to CatSper, the small GTPase Cdc42 distribution in the principal piece is confined to four linear domains and this localization is disrupted in CatSper-null sperm. Cdc42 inhibition impaired CatSper opening, assessed by different approaches including analysis of downstream signaling events and membrane potential recordings. Consequently, Cdc42 inhibition abrogated the increase in intracellular Ca^{2+} and other Ca^{2+}-associated events, resulting in a severe compromise of the sperm fertilizing potential: decreased percentage of sperm undergoing hyperactivation and in vitro fertilization. We also demonstrate that Cdc42 is essential for CatSper function by modulating cAMP production by sAC, providing a new regulatory mechanism for the upregulation of CatSper by the cAMP/PKA-dependent pathway. These results reveal a broad mechanistic insight into the regulation of Ca^{2+} in mammalian sperm, a matter of critical importance in male infertility as well as in contraception.

0531 - TRISTETRAPROLIN (TTP) EXPRESSION IS REQUIRED FOR MAINTENANCE OF THE MAMMARY PROGENITOR CELL POPULATION