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1. Oral communications

Basic Research

OC1-The prognostic significance of E-cadherin in Gastric Cancer: an integrative approach based on patients' cohort and CRISPR-Cas9 engineered cell models

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E-cadherin/*CDH1* dysfunction is a well-established event in GC initiation and progression in nearly 80% of gastric cancers (GC), independently of histological type. While E-cadherin permanent loss is the trigger for diffuse GC (DGC), transient aberrant expression is common along progression in intestinal GC (IGC). DGC has poorer prognosis than IGC and it spreads to the peritoneum, while IGC metastasizes to distant organs. We hypothesize that the timing (initiation vs progression) and mode of E-cadherin loss of function (permanent vs persistent; complete loss vs aberrant) determine the GC pattern of tumour spreading and prognosis and therefore explored the underlying mechanisms. The pattern of E-cadherin expression was analyzed by immunohistochemistry and correlated with clinicopathological features and overall survival (OS) in 284 patients. Permanent (CRISPR-Cas9) and transient (RNAi) E-cadherin depleted cell models representative of DGC and IGC were established and characterized by RNA-sequencing and label-free quantitative proteomics profiling followed by bioinformatics analysis. GC presenting aberrant E-cadherin expression were more often IGC, more advanced, more often spread to distant organs, and displayed poorer prognosis than GC with complete E-cadherin loss or normal E-cadherin expression. Remarkably, GCs with absent/residual E-cadherin expression were more often DGC. Proteomics and transcriptomic profiling revealed that transient and permanent E-cadherin depletion in the DGC model dramatically impairs cell-cell (adherens, tight juntions and desmosomes), and cell-matrix adhesion. The same manipulations in the IGC model led to cadherin-switch and downregulation of adherens junction and cell motility proteins. Our study demonstrates that E-cadherin dysfunction is associated with poor prognosis. Our data supports the hypothesis that E-cadherin transient loss in DGC generates an acute phenotype of cell-cell and cell-matrix adhesion loss that persists and likely prevents spreading to distant sites; while transient or permanent E-cadherin loss in IGC likely triggers cell detachment and expression of alternative cadherins allowing spreading to distant organs.

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OC2-*CYP46A1*- gene therapy improves Machado-Joseph disease in mouse models

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Aims/Context: Machado-Joseph Disease (MJD) is a neurodegenerative disease associated with extensive neuronal death. Defects in brain cholesterol metabolism may contribute to neurodegenerative diseases. Brain cholesterol is almost exclusively synthesized *in situ* and cannot cross the blood-brain-barrier. To maintain the cholesterol homeostasis, superfluous cholesterol is converted into 24S-hydroxycholesterol by the neuronal enzyme cholesterol 24-hydroxylase (*CYP46A1*). The present work evaluated i) whether *CYP46A1* levels are reduced in

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MJD, ii) if *CYP46A1* overexpression could improve MJD, and iii) the mechanisms behind the observed recovery. Methods: *CYP46A1* levels were evaluated in cerebellar extracts of MJD patients and in transgenic MJD mice cerebella. *CYP46A1* overexpression effect was assessed in two MJD mouse models. In the lentiviral-based mouse model, AAVrh10 encoding *CYP46A1* or GFP (control) were injected into the striatum of C57BL6/J mice, and 2 months post-injection the neuronal marker DARPP32 levels and mutant ataxin-3 (mutAtxn3) inclusions' size and number were measured. Transgenic MJD mice were injected into the cerebellum with AAVrh10 encoding *CYP46A1* or GFP and motor performance was evaluated. Then mice's cerebella were analyzed for mutAtxn3 inclusions, Purkinje cell numbers, and cerebellar atrophy. Moreover, *CYP46A1* potential activation of autophagy was evaluated in Neuro2A cells and *in vivo*. Results: Our data indicate that *CYP46A1* cerebellar levels are decreased by 46% in MJD patients and by 29% in MJD mice. *CYP46A1* overexpression reduced DARPP32 loss (48%), mutAtxn3 inclusion number by 59% and their size by 47%. Significant alleviation of motor behavior impairments correlated with mitigation of MJD-associated neuropathology, namely, reduction of Purkinje cell loss (34%) and of cerebellar atrophy (25.40% in lobule X) was observed. Finally, our work demonstrated that *CYP46A1* overexpression induces autophagy (LC3B-II increase and p62 decrease) both in *in vitro* and *in vivo* MJD models. Conclusions: Overall our results demonstrate that *CYP46A1* overexpression improves MJD-associated motor coordination and neuropathology through autophagy enhancement.

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OC3-The role of *CDH1* regulatory noncoding elements for E-cadherin expression

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Introduction: *CDH1* pathogenic germline variants cause Hereditary Diffuse Gastric Cancer (HDGC), in less than half of patients/families fulfilling clinical criteria. *CDH1*-negative cases often display germline *CDH1* monoallelic expression and somatic E-cadherin loss of function. We hypothesized that *CDH1*-negative HDGC may arise due to germline defects in *CDH1* regulatory regions. Therefore, we explored the *CDH1* regulatory network to find expression modifier sequences, controlling *CDH1* expression, that could potentially explain E-Cadherin loss of function phenotypes. Materials and Methods: Capture Hi-C (cHi-C) with a viewpoint in *CDH1* promoter was performed in 5 gastric cancer cell lines, either positive or negative for E-cadherin expression. Mouse embryo reporter assays were used to test a candidate regulatory region by cloning in LacZ-reporter, integration into ColA1 locus of mouse embryonic stem cells, and generation of transgenic mice. Empty-vector mice were used as control for ColA1-driven expression. Tissue-specific β-galactosidase expression was tested in dissected tissues: stomach, esophagus, duodenum, liver (endoderm), heart (ectoderm) and skin (mesoderm). Results: We found evidence that *CDH1* promotor interacts simultaneously with an intergenic region in the short arm of chromosome 2 and an intronic region within *CDH1* intron 2. These interactions were specific of *CDH1*-negative cell lines, highlighting a potential negative regulatory network. We so far tested the regulatory potential of the *CDH1* intronic region and found tissue-specific β-galactosidase expression in endodermal-derived tissues (stomach, esophagus and duodenum), where E-cadherin exerts a primordial function. Conclusion: We found a potential negative regulatory network in gastric cancer cell lines through cis and trans interactions of the CDH1 promoter, and evidence for its tissue-specific regulation in the stomach. These findings suggest a novel mechanism triggering E-cadherin loss of function, worth to be tested in HDGC patients negative for CDH1 germline coding variants.

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OC4-Centrosome positioning and development of ciliopathies: role of the human centrosomal protein TBCCD1

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Aims/Context: Primary cilia are specialized microtubule-based signaling organelles that convey extracellular signaling and cellular polarity into a cellular response. Defects in primary cilia assembly/function cause severe diseases known as ciliopathies, typified by clinical manifestations, as infertility, obesity, brain problems, blindness and kidney cysts. Primary cilia assembly entails centrosome migration to the plasma membrane where a centriole docks, maturates into a basal body (BB), and assembles the cilia axoneme. The human centrosomal TBCCD1 is a critical factor in centrosome positioning previously identified by us. Our aim is to discover the mechanisms/signals required for the correct positioning of the centrosome during cilia assembly, and how these mechanisms, when compromised, are related to ciliopathies. Methods: The proximity-dependent identification (BioID) assay was used to screen for TBCCD1 interactors. Immunofluorescent and super resolution microscopy, as well as Western blot,