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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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in 59% of the patients. The frequency of ε2 allele (associated to a decreased disease risk) was estimated in our sample set with a frequency of 7%. Conclusions: This work is a preliminary study about the frequency distribution of the *APOE* polymorphic ε2, ε3 and ε4 alleles in a cohort of late-onset patients of AD from Northern Iberian regions. The genetic characterization of *APOE* provides a forecast on the landscape of AD risk in these regions based on the haplotype data obtained from *APOE* alleles at SNPs rs429358 and rs7412.

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P23- Sperm DNA damage, active mitochondria and spermatic parameters: influence of the lifestyle, body mass index and age.

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Sperm DNA damage and an altered mitochondrial activity have been related to male infertility. Several common lifestyle (LS) factors, obesity and advanced paternal age may affect sperm quality, although the consequences are not unanimous in the literature. The aim of this study was to evaluate the impact of LS factors (smoking, alcohol intake and exposure to harmful occupational factors), body mass index (BMI) and men's age on sperm (spz) DNA damage, active mitochondria (AM) and spermatic parameters (SP). A total of 149 men (22–52 years old) undergoing infertility investigation collected a sample for routine semen analysis and were asked to complete an anonymous questionnaire about their LS. In 26 individuals, it was evaluated the sperm DNA integrity by Alkaline Comet and TUNEL assays, and the percentage of spz with AM (MitoTrackerTM Red FM dye). Based on the absence or presence of one/more risk factors associated with the LS, two groups of men were formed: with risks (R) and without risks (NR) associated. DNA integrity, spz with AM and SP were compared between individuals R and NR. The SP were also compared between normal weight (NW), overweight (OW) and obese (O) men. The same comparison was made exclusively in individuals NR. We also evaluated the presence of correlations (r) between BMI and DNA damage and spz with AM in individuals R and NR; and between men's age and DNA damage, spz with AM and SP. Individuals NR tended ($p>0.05$) to have sperm samples with less DNA damage (69.8AU vs 73.2AU), less spz with fragmented DNA (4.6% vs 5.3%), more spz with AM (70.3% vs 66.5%) and better SP than the individuals R. In individuals R the higher the BMI, the more DNA damage the sperm samples presented ($r=0.717$, $p=0.030$), although in individuals NR it was only observed a trend ($r=0.661$, $p=0.053$). Individuals O presented worse SP than individuals NW or OW ($p>0.05$), with statistical significance only in individuals NR. Regardless of the LS, the older the men, the more DNA damage the semen samples presented ($r=0.523$, $p<0.01$). Given the results, it is urgent to sensitize the population to adopt a healthy lifestyle and to warn about the decline in semen quality with age.

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P24-Zebrafish: an interesting model to study CDKL5 deficiency disorder

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CDKL5 deficiency disorder is a rare X-linked condition that results in early onset of impaired motor and cognitive skills such as motor rigidity, stereotypical hand movements and deficient language acquisition as well as recurrent seizures. It is caused by mutations in the cyclin-dependent kinase-like 5 (*CDKL5*) gene, which encodes a serine/threonine kinase involved in important neuronal processes such as cell signaling and neuron morphogenesis. CDKL5 is responsible for its autophosphorylation as well as the phosphorylation of its substrates including AMPH, MECP2, MAP1S and ARHGEF2. Although CDKL5 deficiency is a very severe condition, the mechanisms involved in its onset are not clearly understood and existing mouse models do not fully mimic the human phenotype. Thus, the use of alternative models represents a powerful tool to further study CDKL5 deficiency disorder. Zebrafish has been shown to be a suitable biomedical model and shares many physiological processes with human; therefore our objective was to validate the use of zebrafish as a model to study CDKL5 deficiency disorder. Through a comparative *in silico* analysis we confirmed that gene structure of zebrafish *cdkl5* and Cdkl5 substrates appear to be conserved when compared to their human orthologs. The corresponding proteins also show a degree of sequence conservation, particularly Cdkl5 catalytic domains required for phosphorylation. Zebrafish larvae were exposed to PTZ, a seizure inducing drug, total RNA was extracted and qPCR was carried out to investigate expression levels of neuronal marker genes. The results show a downregulation of *mecp2* and an upregulation of *bdnf* after PTZ treatment. Immunohistochemistry of zebrafish brain sections following treatment with PTZ showed a clearly alteration of cdkl5 expression, mostly in telencephalon, comparing with the control. We are presently conducting experiments in zebrafish with morpholinos to suppress Cdkl5 expression in order to investigate the resulting morphological, behavior and molecular changes. In conclusion, our results contribute to validate the use of zebrafish as a suitable model for the study of CDKL5 deficiency disorder mechanisms.

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P25- A BRCA2 intronic variant of uncertain significance alters pre-mRNA splicing

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Context/Aim: Heterozygous germline mutations in the BRCA2 gene are associated with an increased risk for developing breast and ovarian cancers, whereas biallelic pathogenic germline variants in BRCA2 cause Fanconi Anemia. However, a large fraction of genetic alterations found in the BRCA2 gene are classified as variants of uncertain significance (VOUS), precluding an appropriate clinical approach to patients and relatives. The aim of this study was to characterize the splicing pattern and stability of BRCA2 mRNAs in cells from two sisters with cancer that carry a biallelic intronic variant in the BRCA2 gene classified as VOUS. Analysis of DNA crosslink sensitivity was negative for Fanconi Anemia but suggested the presence of genomic instability. Methodology and results: The variant studied localizes in the intron downstream of exon 8, BRCA2: c.681 +5G>C. Using a high precision quantitative RT-PCR assay, based on the digital droplet PCR technique (ddPCR), we show that the great majority of BRCA2 mRNAs produced in the patient cells exclude exon 8. The exclusion of exon 8 results in frameshift and generation of a premature stop codon (PTC) that is expected to drive mRNA to Nonsense Mediated Decay (NMD). We developed an assay based on ddPCR to confirm that BRCA2 transcripts in the patient' PBMCs (peripheral blood mononucleated cells) were indeed targets of NMD. We further found a newly identified exclusion of exon 7 in cells from control donors as well as in the cells from the patients harboring the variant. Conjugated skipping of exon 8 and exon 7 abrogates the frameshift, leading to the expression of an internally truncated BRCA2 protein. Conclusion: Our results suggest that in normal cells the exclusion of exon 7 commits the mRNA to degradation by NMD, whereas in the patients' cells it prevents degradation of the mis spliced mRNA resulting in expression of an abnormal BRCA2 protein. We are currently studying DNA repair function in the patient cells.

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Clinical Research

P27-2q31.1 microdeletion syndrome: mapping the clinical phenotype

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Context: Microdeletions of 2q31.1 region are rare. The clinical phenotype is variable, including intellectual disability (ID), facial dysmorphism and limb defects of varying severity. Less frequently the brain, eyes, heart, and urogenital system may also be affected. Haploinsufficiency of the HOXD gene cluster has been linked to limb anomalies, however the etiology of ID remains unclear. We describe three new cases with 2q31.1 microdeletion, aiming to contribute to the genomic mapping of clinical features for this rare syndrome. Methods: Case 1- 9yo boy, mild ID. Bitemporal narrowing, small palpebral fissures, strabismus, prominent columella, thin upper lip, retrognathia, cupped ears with thickened helices and lobes; 3rd finger camptodactyly, 5th finger clinodactyly; 2nd/3rd toes complete syndactyly; cryptorchidism. Case 2- 6yo girl, mild ID and ADHD. Small palpebral fissures, retrognathia, dysplastic helices; broad hallux, sandal gap and 2nd/3rd toe syndactyly.

Case 3- 9yo girl, mild ID. Microcephaly; triangular face, narrow forehead, underfolded helices, misaligned teeth, high palate; 5th finger clinodactily; 2nd/3rd toes partial sindactily. DNA samples were studied by aCGH (180K CGX-HD). Available parents were studied by FISH analysis. Results/discussion: 2q31.1 microdeletion was identified in all cases: 1-(173550859_176967147)x1; 2-(173120478_176272245)x1 dn; 3-(174436582_175704751)x1. Deletions of HOXD genes result in hand/foot anomalies. Although all our patients have limb defects, only case 1 encompasses HOXD genes, reinforcing the hypothesis that deletion of upstream regulatory elements may also cause limb anomalies. *DLX1/2, RAPGEF4* and *CHN1*, all playing a role in brain development, were suggested as candidates for ID etiology. Only *CHN1* is included in the minimal overlap deleted region in our cases, supporting its key contribution to ID in these patients. *CHN1* participates in the pruning of dendritic arbors. Disruption of these events during circuit maturation and refinement may lead to brain dysfunction and neurological disease. Our cases, with breakpoints defined by aCGH, contribute to the genomic mapping of clinical features for this rare syndrome.

P28-Reproductive Options and Familial Amyloid Polyneuropathy

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Introduction Familial Amyloid Polyneuropathy (FAP), Portuguese type, is a late onset neurodegenerative disease with high penetrance and impressive morbidity. Prenatal diagnosis (PND)