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The abstracts are the author's responsibility.

OP1. EVALUATION OF TUMOUR INFILTRATING LEUKOCYTES AND PERIPHERAL BLOOD T, NK, MONOCYTES AND DENDRITIC CELLS FROM PATIENTS WITH HEPATOCELLULAR CARCINOMA AND CHOLANGIOCARCINOMA

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Introduction: The identification of highly diverse tumour-infiltrating leukocyte (TIL) subsets, and their distinct functions in the tumour niche, has been an important development in oncoimmunology. In this study, we have analysed tumour biopsies and peripheral blood (PB) samples from patients with hepatocellular carcinoma (HCC), and cholangiocarcinoma (CCA). Our aim is to identify potential correlations between the immune system and tumour type at the tumour niche and peripheral level, and their contribution to prognosis.
Materials and methods: A group of 14 patients with HCC (mean age: 64 ± 16.8 years), 5 with CCA (62 ± 18.2 years) and 6 healthy subjects (53 ± 4.4 years) were studied. PB and tumour samples were immediately processed after surgical resection. At tumour and peripheral level we evaluated monocytes/macrophages and myeloid dendritic cells (mDCs); functional subpopulations of T cells (Th/c1, Th/c17); and IFNγ producing NK cells, by flow cytometry.
Results: Biopsies from HCC patients presented a higher percentage of TILs in comparison with CCA biopsies ($p < 0.05$). However, the proportion of CD8 T cells (within the T lymphocyte infiltrate) was higher in CCA ($p < 0.05$). Interestingly, tumour-infiltrating CD4 T cells had a higher percentage of cells producing IFNγ and IL-17 in relation to their PB counterparts ($p < 0.05$), and tumour-infiltrating CD8 T cells presented a higher percentage of cells producing IL-17 in comparison with their PB counterparts ($p < 0.05$). At peripheral level, the percentage of IFNγ+IL-17+ CD4 T and IL-17+ CD8 T cells was decreased in HCC and CCA patients comparatively to the healthy group ($p < 0.05$), as well as, the frequency of mDCs and non-classical monocytes ($p < 0.05$).
Conclusions: The analysis of TIL subsets as well as the PB immune characterization in HCC and CCA allow to understand the role of the inflammatory process in these type of tumours, helping us to identify new clues for the mechanisms by which these tumours grow, evolve and survive under immune attack. Despite being preliminary results, it seems to occur, in both tumours, a specific migration of T cells with the ability to produce IL-17 from PB to tumour microenvironment. IL-17 has been associated with angiogenic and metastatic processes, therefore contributing to an adverse prognosis.
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OP2. HEAD AND NECK CANCER AND SIGNALING PATHWAYS

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Introduction: Head and neck squamous cell carcinoma (HNSCC) presents a high incidence and mortality worldwide. There is an urgent need to develop effective therapeutic approaches to prevent and treat these tumors. The progress of whole-genome technologies has opened new opportunities to explore cancer-associated pathways with therapeutic applications. This study aimed to perform a genome-wide characterization of HNSCC patients and to identify the most common altered signaling pathways and genes related to the different anatomic susbsites and stages of the primary tumor as well as with the development of metastasis/relapses.
Materials and methods: The genomic characterization of 102 HNSCC cohort was performed using array comparative genomic hybridization technique, Agilent oligonucleotide microarray 4x180K. Copy number variation data was analyzed using Matlab, R programming language and SPSS. A pathway enrichment analysis was performed, the overrepresented pathways and the respective genes altered in our cohort were determined. The association between gene alteration and staging, location of the primary tumor and metastasis/relapses development was tested.
Results: With this whole genome approach, we detected imbalances in all chromosomes; however, it was possible to verify that the most common deletions and amplifications were observed in specific chromosomal regions. Chromosomes 3, 5, 8 and 11 were the most frequently altered in our cohort. The two most statistically significant pathways associated with the amplified and deleted genes were Cell cycle and PI3K-Akt signaling pathways, and Cytokine-cytokine receptor interaction and Ubiquitin mediated proteolysis signaling pathways, respectively. From the cell cycle and PI3K-Akt signaling pathways we verified statistically significant amplification of *MYC* and *RBL2* genes in the different tumor anatomic subsites. From the PI3K-Akt signaling pathway we found that *MCL1* and *COL9A2* genes seem to be related to the development of relapse/metastasis (OR > 4.7 and 2.7 respectively).
Conclusions: These highlighted pathways are commonly activated in cancer. The correlation between molecular and clinic-pathological data has the power to identify specific genes that could help to understand the disease progression and, consequently that could have prognostic value. Further studies are required to validate the role of these genes and signaling pathways in the tumor evolution and behavior.

OP3. ALDEHYDE DEHYDROGENASES AS POTENTIAL BIOMARKERS IN MYELOID NEOPLASIAS

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Introduction: Aldehyde dehydrogenases (ALDH) are critical to the protection against toxic aldehydes and have been associated with multiple diseases. Myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) are two myeloid neoplasias with a complex multistep development involving abnormal differentiation, cellular proliferation, and apoptosis. Since ALDHs are involved in some of these biological processes, the deregulation of these enzymes may influence MDS and AML development. This study aimed to evaluate the gene expression levels of ALDHs in patients with MDS and AML in order to verify their potential as a biomarker for the diagnosis and/or prognosis of these diseases.
Materials and methods: To this end, we analyzed the gene expression levels of *ALDH1A1*, *ALDH1A2*, *ALDH1A3*, *ALDH1B1*, *ALDH1L1*, *ALDH1L2*, *ALDH2*, *ALDH3A1*, *ALDH3A2*, *ALDH3B1*, *ALDH3B2*, *ALDH4A1*, *ALDH5A1*, *ALDH7A1*, *ALDH16A1*, and *ALDH18A1*. The ALDH expression levels were analyzed in 54 patients, 34 MDS, and 20 AML, and 34 healthy controls, using RT-PCR and the differentially expressed genes were quantified by qPCR. The statistical analysis was carried out by uni- and multivariate tests and ROC analysis. A value of $p < 0.05$ was considered significant.
Results: The results indicate that *ALDH3A2*, *ALDH3B1*, *ALDH4A1*, and *ALDH18A1* had a differential expression among the study groups and were posteriorly quantified by real-time PCR. MDS and AML patients showed higher median expression of *ALDH3A2* [MDS: 1.93 interquartile range (IR) 1.28; $p < 0.001$; AML: 1.51 IR 0.99; $p = 0.008$)] and *ALDH4A1* (MDS: 0.18 IR 0.47; $p = 0.011$; AML: 0.16 IR 0.78; $p = 0.012$) in comparison with controls (*ALDH3A2*: 0.4624 IR 1.53 *ALDH4A1*: 0.0388 IR 0.12). The expression of ALDH3B1 was higher in MDS patients (1.64 IR 1.39) than in AML patients (0.45 IR 0.47; $p < 0.001$) and controls (0.35 IR 0.51; $p < 0.001$). Additionally, patients with MDS and AML with myelodysplasia-related changes (AML-MRC) did not express ALDH18A1. ROC curve analysis showed that *ALDH3A2* ($p = 0.001$), *ALDH3B1* ($p < 0.001$), and *ALDH4A1* ($p = 0.012$) were able to discriminate MDS patients from controls. Moreover, *ALDH3A2* was the only isoform with diagnostic value for AML patients.
Conclusions: ALDH isoforms have differential expression patterns in MDS and AML patients when compared with controls and with each other, and can be good diagnostic biomarkers of these diseases. However, further studies are needed to prove the potential of these enzymes as diagnostic/prognostic biomarkers.

OP4. UNCOVERING MIRNA-MEDIATED CIS-REGULATION IN COMMON CANCERS

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Genome-wide association studies (GWAS) identified a large number of risk loci to be associated with common complex diseases. The difficulty remains however in pinpointing the causative genetic variants within those risk loci, and in uncovering the underlying molecular mechanisms. Increasing evidence supports a cis-regulatory role for most of the risk-associated single nucleotide polymorphisms (SNPs), which mostly lie in non-coding regions. The few functional studies carried in cancer GWAS loci have mainly investigated the role of the putative causal variants in altering transcription factor binding. Nevertheless, other mechanisms of cis-regulation exist, such as microRNA (miRNA) regulation. In this mechanism, SNP alleles can either create or destroy target sites, if located at the seed region of miRNAs or the target sequence of regulated genes, as well as regulate the levels of expression of the miRNAs themselves. Here, we developed a bioinformatic analysis pipeline for evaluating the contribution of allele-specific miRNA regulation in cancer risk, using breast cancer (BC) as a model disease. We selected the 223-haplotype tagging SNPs identified in BC GWAS and queried their proxies in high linkage disequilibrium. SNPs were filtered based on 1) their location in either miRNA genes and/or miRNA target genes and 2) previous evidence of cis expression

quantitative trait loci (cis-eQTL) and differential allelic expression (DAE), an indication that the gene is being cis-regulated. Selected SNPs were screened through the TargetScan algorithm, modified to analyse sequences carrying SNP alleles. Finally, results were short-listed for miRNAs with evidence of expression in breast tissue. Surprisingly, none of the SNPs mapped to miRNA genes, suggesting that altered miRNA biogenesis and target binding are unlikely mechanisms involved in BC risk. Of the SNPs located in the 3' un-translated region of target genes, we found 24 that were predicted to alter the miRNA-mRNA binding stability in nine genes. These included rs4245739 in *MDM4* and rs11540855 in *ABHD8*, previously shown to cause allele-specific miRNA binding, which validates our predictions. To our knowledge, this is the first systematic study looking into the global role of miRNA regulation in BC risk, further improved by the use of cis-eQTL and DAE mapping in breast tissue. This method provides a new opportunity to explore the role of miRNA cis-regulation in the risk loci identified for multiple common cancers.