PROTEIN MARKERS OF PROGRESSION RISK IN PATIENTS WITH SQUAMOUS CELL VULVAR CARCINOMA

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Introduction The application of the concept of tumour-specific markers to assess a possible disease outcome and to choose appropriate treatment options, is still obstructed by the limited knowledge on vulvar carcinoma (VC) biology. We aimed to identify protein markers of VC that would be indicative of a tumour that is more likely to progress.

Material and methods Primary tumour samples from 28 patients with early stage squamous cell VC and 14 samples of normal vulvar tissue were studied using iTRAQ analysis. The results obtained for tumour samples of VC patients that progressed during 8–12 years of follow-up period (‘progVC’, n=14) were be compared to those obtained for samples of patients who were disease-free at the time of last observation (‘d-VC’, n=14). The differentially expressed proteins were subsequently validated using targeted proteomics methods (PRM) and immunohistochemistry using a larger sample set.

Results and discussions 5510 proteins were identified in the analysed samples. Gene Ontology (GO) analysis of the proteins differentially expressed in progVC and d-VC tumours proteins indicated an immune response as the most over-represented GO category in this sample comparison. The top 47 candidate markers were chosen for further validation. Correlation of the validation results with clinical parameters of the enrolled VC patients indicated that HMGA2, ANO1, PRTN3, UBE2C, AB13BP and PRELP should be considered as potential protein markers for the prediction of progression in VC patients.

Conclusion Our findings provide evidence showing that deregulation of eight proteins’ abundance is significantly associated with aggressive phenotype of VC. Their immunohistochemical assessment hold promise as a patient stratification tool.

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THE ALTERNATIVE NF-κB PATHWAY IN COLORECTAL CANCER: FROM GENETIC POLYMORPHISMS THROUGH MRNA TO PROTEIN LEVELS

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Introduction In colorectal cancer (CRC), there is scarce information regarding the alternative NF-κB pathway which regulates different aspects of immune functions.

Material and methods Formalin-fixed paraffin-embedded (FFPE) tissue samples from 116 patients with CRC were analysed with IHC to assess NF-κB2, Bcl3, RelB and NIK protein expression in tumour (T), adjacent non-neoplastic (adjNN) and non-neoplastic (NN) tissues. Gene expression was assessed with real time RT-PCR. Genotyping for BCL3 rs8100239, NFKB2 rs7897947 and rs12769316 and NIK rs7222094 was performed with High Resolution Melting Curve analysis.

Results and discussions NF-κB2 and NIK proteins were found mainly in the cytoplasm, whereas Bcl3 and RelB exhibited cytoplasmic and nuclear expression. T cells exhibited higher cytoplasmic expression of each of the four proteins compared to adjNN and NN tissue from a more distant to the tumour site (p=0.000, for all proteins). Protein levels were variably correlated with mRNA levels. The four molecules presented similar mRNA levels between T and NN tissue except for RELB (p=0.002). Particular protein and mRNA levels and SNPs provided prognostic value regarding DFS and OS. Patients who were rs8100239 A carriers (HR=0.356, 95% CI: 0.148–0.862, p=0.022) or exhibited high RELB T cytoplasmic or nuclear expression (HR=0.276 95% CI: 0.104–0.732, p=0.010 and HR=0.260, 95% CI: 0.081–0.834, p=0.023, respectively) presented with improved OS in both single and multiparametric analysis. Moreover, a longer time to progression was noted for patients who were carriers of the rs7897947 G allele (HR=0.362 95% CI: 0.136–0.960, p=0.041) or the rs8100239 A allele (HR=0.292 95% CI: 0.135–0.631, p=0.002). Similarly, patients with high T mRNA levels of each molecule under study or with high cytoplasmic T RelB (HR=0.165 95% CI: 0.060–0.459, p=0.001) or cytoplasmic T NIK (HR=0.133 95% CI: 0.045–0.395, p=0.000) or nuclear T Bcl3 expression (HR=0.329 95% CI: 0.114–0.947, p=0.039) had longer DFS.

Conclusion The alternative NF-κB pathway is deregulated in CRC. Selected genotypes, mRNA and protein levels of NF-κB2, Bcl3, RelB and NIK appear to have prognostic value.

HIGH-THROUGHPUT DRUG SCREENING TO IDENTIFY THERAPIES REVERSING ABERRANT CELL DIFFERENTIATION IN AML

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Introduction Acute myeloid leukaemia (AML) is a malignancy characterised by impaired cell differentiation and uncontrollable accumulation of immature myeloid progenitor cells in the bone marrow. Although most patients respond to the first line chemotherapeutic treatment, the majority of patients eventually relapse and therefore the overall survival from AML remains very poor. A therapy based on inducing the immature leukemic cells to differentiate may turn AML into a curable disease, as has been shown in the case of Acute Promyelocytic Leukaemia (APL), the M3 subtype of AML. However, AML is a heterogeneous disease and we still lack knowledge of how different genetic alterations disturb normal hematopoiesis and how the cell differentiation blockade may be lifted in the various subtypes of AML. Thus, our aims are 1) to identify therapies reversing aberrant cell differentiation in AML by using flow cytometry-based high-throughput drug screening 2) to...
establish links between molecular subtypes of AML and responsiveness to identified cell differentiating therapies.

**Material and methods**
Our approach is to explore drug-induced cell differentiation in primary samples from AML patients and genetically engineered AML mouse models using high-throughput flow cytometry. Specifically, we characterise samples by their expressed cell differentiation-related cell surface markers with and without drug treatment to identify compounds capable of inducing cell differentiation *ex vivo*. The primary AML patient samples are also profiled by exome and RNA-sequencing.

**Results and discussions**
Final results with a Flt3/Npm1 – mutated mouse model suggest that several compounds elicit cell differentiation-state specific responses, some of which could be indicative of a dynamic shift towards cell differentiation. For example, treatment with mTOR-kinase inhibitors sapanisertib and virstusertib resulted in a concentration dependent increase of differentiating cells marked by CD11b expression and decrease of stem cells marked by CD34 expression.

**Conclusion**
We have established a high-throughput flow cytometric screening platform to identify drugs that are able to induce differentiation in primary AML samples and genetically engineered mouse models of AML. By integrating drug response data with genetic background information from samples we aim to reveal novel genotype-phenotype relationships in the level of cell differentiation that could eventually be translated into clinically relevant biomarkers and treatment options for AML patients.

**PO-515**

**TRANSCRIPTOME ANALYSIS IDENTIFIED ALCAM EXPRESSION AS POTENTIAL BIOMARKER TO LSCC PATIENT’S OUTCOME**

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**Introduction**
Laryngeal squamous cell carcinoma (LSCC) is one of the most incidence tumours in the world, especially in developing countries, such as Brazil. The main risk factors for LSCC are tobacco and alcohol consumption and it usually occurs in patients older than 60 years. Similarly to other head and neck tumours, LSCC is a major health problem because of poor prognosis and slight improvement in the five-year survival during the past four decades. Therefore, our objective was to better understand the LSCC transcriptome, identifying possible prognosis biomarkers and novel therapy targets.

**Material and methods**
Was carried out a gene expression profile analysis using the Human Exon 1.0 ST microarray chip. To this end, the expression profile of 14 tumour tissues were compared with 12matched nonmalignant mucosa. A multivariate analysis applying Cox regression was performed on all univariate significant variables.

**Results and discussion**
Transcriptome analysis pointed out 817 differentially expressed genes (DEG), 315 of which with overexpression in tumour and 502 underexpressed when compared with matched nonmalignant mucosa. Performing a survival analysis across all overexpressed DEG, 24 genes were related to patients survival (*ACOX1*, *ACVR1*, *ADH7*, *AGFG2*, *ALCAM*, *BTBD11*, *CDK14*, *CYP2C19*, *GBP6*, *GLTO*, *GNG4*, *LOX*, *LYPD68*, *ME1*, *NPEPPS*, *ODC1*, *PMM1*, *PTGR1*, *SERNP1*, *ST3GAL4*, *TDP52L1*, *ZDHHC13* and *ZNF750*). The prognostic values of these 24 genes were, then, validated in an independent LSCC data set of The Cancer Genome Atlas (TCGA) and the expression data, patient’s clinic and pathological features were analysed in Log-rank Test. Perineural invasion, commitment of surgical margins and expression of *LOX*, *ALCAM*, *BTBD11* and *LYPD68* showed p<0.05 and were selected to multivariate analysis applying Cox regression model. After age and tumour stage-adjustment, perineural invasion, commitment of surgical margins and *ALCAM* expression were classified as independent prognostic factors to LSCC patients. LSCC-patients displaying *ALCAM* high expression presented 4.5-fold increased death risk. *ALCAM* gene encodes the activated leucocyte cell adhesion molecule protein, also known as CD166, which is a member of a subfamily of immunoglobulin receptors. This protein binds to T-cell differentiation antigen CD6, and is implicated in the processes of cell adhesion and migration.

**Conclusion**
*ALCAM* expression might be an independent prognosis biomarker to LSCC patients. Furthermore, *in vitro*, *in vivo* and pre-clinical analyses must be performed to reinforce this hypothesis.

**PO-516**

**GENES OF INFILTRATED IMMUNE CELLS ARE PROGNOSIS BIOMARKERS IN SPECIFIC SUBTYPES OF COLORECTAL CANCER**

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**Introduction**
Colorectal tumours are composed of mixed tissues, including tumoral and stromal cell types. The functional communication between these cell entities defines the biology of the tumours and eventually influences disease progression and patients’ prognosis. Recently, the first bona fide consensus molecular subtypes of colorectal cancer have been defined, each of them showing a specific immune-cell composition. CMS1 comprises hypermutant tumours with better prognosis associated to a higher immune infiltration. CMS2 tumours are characterised by a high CIN and strong WNT/MYC pathways activation. CMS3 have activated pathways related to metabolism. CMS4 tumours are more stromatic and showed the worst survival. In this work, we aim to assess the immune cell infiltration in the tumour as a prognosis indicator for colorectal cancer as well as to understand the molecular biology behind this crosstalk, while stratifying tumours by molecular subtype.

**Material and methods**
Our discovery series includes 100 paired normal and stage II MSS tumour samples. For validation purposes, public GSE39582 and TCGA series have been used. Using gene expression data, tumours have been classified into CMS subtypes using CMSclassifyer R package. We were estimating tumour immune cell infiltration (R library MCPcounter) and calculating the immunophenoscores. GSEA has been performed to make a functional enrichment analysis. To assess the utility as prognosis biomarkers of genes expressed in the immune compartment, a cox model were fitted.