**Introduction**

Esophageal cancer (EC) is the eighth most frequent cancer and the sixth leading cause of cancer-related deaths worldwide, with squamous cell carcinoma (ESCC) corresponding to 80% of the cases._

**Material and methods**

Three CpG sites of \( \text{SPRR3} \) (A, B for promotor region and C for 5'UTR region) were analysed by pyrosequencing in esophageal cancer cell lines (TE-1, TE-13 and OE21) treated with the demethylating agent decitabine, and in tumour and matched normal surrounding mucosa from patients with ESCC. RT-qPCR was performed to evaluate \( \text{SPRR3} \), DNMT1, DNMT3A and DNMT3B expression in the same samples.

**Results and discussions**

Detectable treatment was able to induce \( \text{SPRR3} \) demethylation and reestablished esophagin expression in the three cell lines tested. The methylation of all CpG sites analysed was significantly higher in tumours in comparison with the adjacent tissues (p<0.0001 for the three CpG sites analysed). The ability of \( \text{SPRR3} \) methylation to distinguish the adjacent mucosa from tumour samples using ROC curve analyses was significant for each of the CpG sites examined (A, sensitivity 93.10% and specificity=86.21%, p<0.0001; B, sensitivity=88.24% specificity=76.47%, p<0.0001; C, sensitivity=82.35% and specificity=73.53%, p<0.0001). We observed an inverse correlation between \( \text{SPRR3} \) expression and p53 expression, and p<0.0001). We observed an inverse correlation between \( \text{SPRR3} \) mRNA expression and all CpG sites evaluated (A and B p<0.0001; C p=0.0007). In order to investigate the mechanisms that could be involved in \( \text{SPRR3} \) hypermethylation, we evaluated DNMT expression (higher expression in tumour in comparison with the normal surrounding mucosa, p<0.0001 DNMT1, p=0.0005 DNMT3A and p<0.0001 DNMT3B) which are inversely correlated with \( \text{SPRR3} \) expression (DNMT1 p=0.0013, DNMT3A p=0.0108 and DNMT3B p<0.0001). We observed an inverse correlation between \( \text{SPRR3} \) methylation and DNMT3B mRNA expression for all CpG sites evaluated except for DNMT3A and Site C.

**Conclusion**

Thus, our data provide evidences that DNA methylation induced by DNMT3B is a possible mechanism involved for \( \text{SPRR3} \) silencing in ESCC.

**Introduction**

Chondrosarcoma is the second most common bone malignancy. 50% to 70% of these tumours have a mutation in isocitrate dehydrogenase (IDH)—1 or IDH2, enzymes that are involved in the citric acid cycle. The specific mutations in IDH1/2 lead to the formation of the oncometabolite (D)–2-hydroxyglutarate (D2HG), causing changes in for instance the metabolism and epigenetics of cells. D2HG inhibits \( \alpha \)-ketoglutarate (\( \alpha \)KG) dependent enzymes such as histone and DNA demethylases, leading to a hypermethylation phenotype. Nowadays, surgery is the only treatment option for these patients which underlines the need for the development of novel therapeutic strategies. We here explore whether the underlying alterations caused by the mutations in IDH1/2 can be used as a target for therapy of unresectable chondrosarcoma.

**Material and methods**

To identify targets, we used an epigenetic compound library including 128 inhibitors of epigenetic enzymes such as histone deacetylases (HDACs), histone acetyltransferases (HATs) and DNA methyltransferases (DNMTs). In addition, we performed a siRNA screen focused on 149 genes encoding several epigenetic regulators. The screens were performed in (up to) three chondrosarcoma cell lines to exclude cell line specific effects. JJ012 and SW1353 harbour an IDH1 and IDH2 mutation, respectively, while CH2879 is wildtype for both genes. Furthermore, screens were combined with AGI-5198 (IDH1 mutant inhibitor) or D2HG, to identify synthetic lethal interactions with IDH1/2 mutations. Interesting hits were selected for further study in validation screens and additional chondrosarcoma cell lines.

**Results and discussions**

In both screens, no synthetic lethal interactions with IDH1/2 were identified, since there was no significant difference in effectivity of compounds or siRNAs between mutant and wildtype cell lines, and combination with AGI-5198 or D2HG did not rescue or sensitize cells. The epigenetic compound screen identified several interesting compound classes, including PARP, HDAC and DNMT inhibitors. The efficacy of these compounds was confirmed in the other chondrosarcoma cell lines. The epigenetic siRNA screen did not identify single genes with a major effect on cell viability.

**Conclusion**

Epigenetic changes in chondrosarcoma seem to be promising therapeutic targets. However, this vulnerability is not correlated with the IDH1/2 mutation status. Further research is needed to explore why these specific compounds inhibit the growth of chondrosarcoma cell lines in vitro and to confirm compound efficacy in vivo.
that can be used are good noninvasive biomarkers detected in liquid biopsy samples, such as liquid-based cervical cytology (LBC) samples. However, a few studies have been carried out in these kind of samples to detect the expression profile of miRNAs in cervical cancer precursor lesions. Furthermore, the most of the studies used a small samples or evaluated a few groups of miRNAs. The analysis of miRNAs profile would allow to open diagnostic testing arsenal for the screening of Cervical Intraepithelial Neoplasia (CIN). In view of the above, there is a need for technical standardisation for the analysis of miRNA expression in LBC samples considering the poor evidence in the literature that used LBC samples for the analysis of miRNA expression. Nevertheless, the aimed of this study was to identify housekeeping’s for analysis of miRNA expression in LBC samples.

**Material and methods** Expression of U6, hsa-miR-16 RNU-44, 47, 48 and 49 was measured by Reverse Transcriptase Quantitative PCR (RT-qPCR). Reference genes expression values were normalised to the reference using the comparative CT method (2−ΔΔCT)

We used one common software, namely NormFinder, to analyse expression stability of the six selected genes. This software candidate reference genes by calculating their stability values, with lower values indicating more stable genes.

**Results and discussions** The stability values calculated using NormFinder to six housekeeping was (U6=0.787; miR-16=9.728; RNU-44=7.912; RNU-47=3.234; RNU-48=5.132; RNU-49=1.716) and the standard error (U6=2.127; miR-16=2.127; miR-16=9.728; RNU-44=7.912; RNU-47=3.234; RNU-48=5.132; RNU-49=1.716) and the standard error (U6=2,127; miR-16=9,728; RNU-44=7,912; RNU-47=3,234; RNU-48=5,132; RNU-49=1,716) and the standard error (U6=2,127; miR-16=9,728; RNU-44=7,912; RNU-47=3,234; RNU-48=5,132; RNU-49=1,716) and the standard error (U6=2.127; miR-16=9.728; RNU-44=7.912; RNU-47=3.234; RNU-48=5.132; RNU-49=1.716). Thereby, we can verify that U6 was the gene that demonstrated to be the best housekeeping, this because it presented smaller variation in the expression in relation to the other genes tested. Similar data were demonstrated in the literature. However, it was the first time that it was performed with liquid-based cervical cytology samples.

**Conclusion** We conclude that the small nucleolar RNA transcript U6 was the best reference gene.

**DNA METHYLATION OF ADAM23 IS NEGATIVELY ASSOCIATED WITH HAEMATOGENOUS SPREAD IN BREAST CANCER**

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**Introduction** Circulating tumour cells (CTCs) are associated with metastatic potential in a variety of human cancers. Although DNA methylation of cancer-related genes is a promising diagnostic and prognostic tool, it was rarely studied in relation to the presence of CTCs. ADAM23 gene, member of ADAM (a disintegrin and metalloproteinase domain) family, is involved in various biological events such as cell adhesion, fusion, migration, membrane protein shedding and proteolysis. The aberrant DNA methylation of ADAM23 was associated with expression silencing in breast, gastric, brain and other tumours. In our study we investigated the role of ADAM23 methylation in hematogenous spread of breast cancer (BC).

**Material and methods** To detect CTCs, we analysed expression levels of KRT19, SLUG and TWIST genes in the CD45-enriched peripheral blood samples of 203 primary BC patients. The methylation levels of ADAM23 were quantified by pyrosequencing in formalin-fixed, paraffin-embedded tumour tissues. The hypermethylation cut-off was defined as mean ±2 standard deviation of DNA methylation in healthy breast tissues. Protein expression was evaluated in the same material by ImmunoReactive Score (German IRS system), based on the proportion of positive cells and the staining intensity of the nuclei or cytoplasm. A logistic regression was used to determine the effect of studied variables on presence of CTCs in peripheral blood.

**Results and discussions** CTC positivity (epithelial or mesenchymal markers) was identified in 23% of patients (16% mesenchymal, 9% epithelial and 9% both). ADAM23 hypermethylation (DNA methylation ≥10%) did not occur in mesenchymal CTC positive patients, while it was present in 28.8% of mesenchymal CTC negative patients (p=0.001). Among studied variables, the multivariate analysis identified low ADAM23 methylation level (p=0.004) and high ki67 proliferation index (p=0.005) as two most significant predictors of mesenchymal CTCs. It was shown recently that although ADAM23 inhibition enhanced invasive in breast cancer, it was not sufficient to trigger metastasis (Costa et al., 2015).

**Conclusion** Our data indicate possible involvement of ADAM23 in hematogenous spread of BC cells. Better understanding of reversible epigenetic regulations hold promise for development of novel therapeutic approaches.

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**MECHANISTIC INVESTIGATION OF EPIGENETIC MODIFICATIONS INDUCED BY METABOLIC CHANGES IN AML**

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**Introduction** Acute Myeloid Leukaemia is the most common and aggressive adult leukaemia with a high frequency of relapse and mortality. This is likely due to the failure to eliminate the malignant stem like population (LSCs) composed by low cycling cells that give rise to highly proliferating blast type progenitors. Although the currently available chemotherapeutic agents efficiently eradicate blasts, LSCs can be retained and cause relapse. Therefore, new therapeutic strategies aimed to specifically eradicate them is urgently needed.

These insights led to an increasing interest in understanding the molecular mechanisms governing LSCs biology. Along this line, recent reports (Raffel et al. Nature 2017) highlight the crucial role of Branched Chain Amino Acid (BCAA) catabolism in LSCs. Indeed, BCAA Transaminase 1 (BCAT1) is highly expressed in LSCs and led to reduction of alpha-ketoglutarate (aKG) levels, with consequent impairment of aKG-dependent dioxygenases activity, such as TET1/2, and increase in DNA methylation, resembling AML with TET or an IDH mutation. Moreover, BCAT1 overexpression is associated with poor overall survival in patients which have no TET or IDH mutation.