CONTRIBUTION OF ELEVATED MIR-146B-5P TO Methylaton DEREGULATION OF MIRNAS

Introduction Papillary thyroid carcinoma (PTC) represents 80% of thyroid cancers and ranks among the five most common cancers in many countries including Kuwait. MicroRNAs (miRNAs) are endogenous non-coding RNAs that contribute to the pathogenesis of multiple cancers by interfering with many biological processes. PTC specific miRNAs can have clinical utility as diagnostic and prognostic markers or targets to manipulate the progression of the disease. This study aimed to investigate the diagnostic potential and the biological function of miR-146 in PTC development.

Material and methods The expression of miR-146b-5p was assessed by Quantitative real time PCR in different variants of PTC (113 samples), 20 cases of benign thyroid tumours and 10 cases of thyroid hyperplasia. miR-146b-5p inhibitor was transfected into primary thyroid cultured cells and the activity of 10 signalling pathways were assessed by luciferase assay. The obtained results were confirmed by immunofluorescence staining and confocal microscopy. The effect of mir-146b-5p on cancer cells growth was assessed using apoptosis assay and flow cytometry. Ethical approval to conduct this study was obtained from Kuwait Ministry of Health and Kuwait University Health Sciences Centre ethics committee.

Results and discussions The real time PCR results followed by analysis with the Receiver operating characteristic curve showed that miR-146b-5p significantly discriminates classic PTC from other PTC variants and benign tumours (86% sensitivity and 73% specificity). Inhibition of miR-146b-5p activated the JNK/AP1 pathway and increased the number of apoptotic cells. Active Phospho-JNK was detected by immunofluorescence in the nuclei of tumour cells in PTC cases with low miR-146b-5p level but not detected in tissues with high miR-146b-5p. These results confirm that miR-146b-5p inhibits activation of the JNK/AP1 pathway in vitro and in vivo. Considering the importance of the JNK pathway in facilitating the stress mediated cell death, inhibition of this pathway can be an attempt of the cancer cells to overcome the stress response and maintain survival.

Conclusion High level of miR-146b-5p distinctively characterises classic PTC. miRNA-146b-5p contributes to PTC carcinogenesis by regulating the stress kinase pathway in thyroid cells.

INVESTIGATING THE EPIGENETIC CHANGES UNDERLYING COMBINATION TREATMENT OF ACUTE PROMYELOCYTIC LEUKAEMIA WITH ALL-TRANS RETINOIC ACID AND ARSENIC TRIOXIDE

Introduction Acute promyelocytic leukaemia (APL) results in the interrupted differentiation of granulocytes. Combination induction therapy using all-trans retinoic acid (ATRA) and arsenic trioxide (ATO) is the most effective treatment of APL. DNA methylation and histone modifications are epigenetic mechanisms of gene regulation aberrant in APL. While ATRA treatment of APL NB4 cells restores granulocyte differentiation and induces significant genome-wide changes in histone modifications, it does not affect the aberrant DNA methylation. Similarly, ATO has comparable effects. Therefore, we hypothesise that the increased effectiveness of ATRA and ATO when combined is due to epigenetic changes at both the histone and DNA methylation level, resulting in profound changes in gene expression.

Material and methods We identified the concentration of ATRA and ATO in combination that induced enhanced differentiation and cell death of NB4 cells in comparison to single agent treatment through fluorescence activated cells sorting (FACS) of CD11b and 7-AAD labelled cells at 72 hours and 96 hours post treatment termination. Quantitative PCR (qPCR) was performed to determine the expression levels of a panel of genes after 72 hours of treatment and 96 hours post treatment termination.

Results and discussions ATRA and ATO in combination induced significant differentiation and cell death of NB4 cells after 72 hours. Interestingly, only the combination-treated cells maintained differentiation when treatment was terminated for 96 hours. Additionally, combination treatment significantly increased the expression of several genes important in APL compared to single agent treatment after 72 hours as well as 96 hours post treatment termination. Chromatin immunoprecipitation (ChIP) is currently being utilised to investigate the enrichment of chromatin with acetylated and methylated histones, and qPCR was performed to assess the enrichment of several genes of interest. Finally, bisulfite pyrosequencing is being performed to characterise the specific methylation pattern of these genes of interest.

Conclusion ATRA and ATO in combination synergistically induces the differentiation of APL NB4 cells. Together, the ChIP and bisulfite pyrosequencing experiments will enable us to characterise the effects of combination treatment at the epigenetic level. Ultimately, understanding the epigenetic changes induced by ATO and ATRA synergy can help improve treatment in the clinic.

METHYLATION DEREGLUATION OF MRNAS PROMOTERS IN BREAST CANCER IN VERY YOUNG WOMEN

Introduction Breast cancer has the highest incidence rate of all cancers in women worldwide. Although early breast cancer generally has an excellent prognosis, breast cancer in young women is associated with a high risk of systemic disease at long-term follow-up. Epigenetic modifications were reported to play an important role in many disease onsets. MiRNAs not only function as a part of epigenetic machinery, but are also epigenetically modified by DNA methylation. The aim of the current study is to analyse the methylation alterations of CpG associated to miRNA encoding genes in breast cancer tumours occurring in very young women (BCVY) (<35 years old) and older ones (BCO) (>45 years old).
Material and methods We analysed DNAmethylation of 1264 genes encoding miRNAs in 26 BCVY samples and 15 samples from BCO using the ‘Illumina Infinium MethylationEPIC BeadChip’ array. Methylation differences were assessed using Wilcoxon Rank Sum test. Differences observed were validated using two independent populations: Flower et al 2015 and The Genome Caner Atlas (TCGA) methylation datasets. Expression of miRNAs regulated by CpG sites differently methylated were analysed in a meta-analysis using data from ClarionD array, miRNA expression published data from Peña-Chilet et al 2014 and TCGA gene expression data. We perform an expression validation study by real time-PCR of miRNAs differently methylated and expressed in BCVY comparing with older ones.

Results and discussions We identified 193 significantly differently methylated CpG sites that were regulating genes encoding miRNAs. The hypomethylated CpGs were localised in islands and regions away from them (opensea) were mainly hypermethylated. We could validate a total of 10 methylation probes regulating miRNAs in the methylation validation data sets. Finally, the miRNAs mir-9~1, mir-184, mir-551b and mir-196a-1 were significantly differently methylated and de-regulated in BCVY in comparison with BCO.

Conclusion We identified a differential miRNA methylation profile in BCVY. Most of the miRNAs differently methylated between breast cancer age groups were previously described with aberrant methylation in cancer. Additionally, some of them presented significant different expression. The present study includes for the first time an analysis of methylation of miRNA encoding genes using the EPICarry in a large cohort of breast cancer samples occurring in young women. The four identified miRNAs with different methylation and expression in BCVY may be a promising epigenetic biomarkers in BCVY risk.

PO-374 LINC00152 LONG NON-CODING RNA PROMOTES THE PROLIFERATION OF SW480 COLON CARCINOMA CELLS THROUGH REGULATION OF CELL CYCLE AND WNT SIGNALLING PATHWAY

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Introduction Long non-coding RNAs (lncRNAs) contribute to different cancers including colorectal cancer (CRC). Previous studies have shown altered LINC00152 expression in CRC, but the detailed mechanism of its effects during CRC development and progression is not well studied. We aimed to study the effects of LINC00152 silencing on the cell cycle regulation and whole transcriptome in colon carcinoma cells. We also analysed the DNA methylation alterations caused by LINC00152 knockdown.

Material and methods LINC00152 were silenced in SW480 colon carcinoma cells using Stealth siRNAs. Flow cytometric cell cycle analysis was performed using propidium-iodide DNA staining. Cyclin D1 protein expression was detected using flow cytometry after labelling with anti-cyclin D1 antibody. The effect of LINC00152 silencing to genome-wide gene expression was studied on Human Transcriptome Array 2.0 microarrays. DNA methylation alterations after LINC00152 knockdown were evaluated using Reduced Representation Bisulfite Sequencing (RRBS) method using NextSeq500 device.

Results and discussions Silencing of LINC00152 significantly suppressed cell growth compared to negative control cells (p<0.05). LINC00152 knockdown caused approximately two-fold increase in apoptosis (48 hour: si-NEG: 4%; si-LINC00152: 8%; 72 hour: si-NEG: 11%; si-LINC00152: 23%) (p<0.05). In parallel with the growth inhibitory effect, silencing of LINC00152 could reduce cyclin D1 expression already after 48 hours, however, significant decrease was detected after 72 hours in the LINC00152 siRNA-treated cells (si-NEG MFI=0.70 and si-LINC00152 MFI=0.56) without attenuation of phospho-S6 protein. Whole transcriptome microarray analysis of LINC00152 silenced cells revealed significant under-expression of genes with oncogenic and/or metastasis promoting function (e.g. STC1, YES1, HES1, KLK6, PORCN) and up-regulation of tumour suppressor genes (e.g. DKK1, PERP) (FDR p<0.05, absolute value of logFC >1). Using RRBS, DNA methylation alterations after LINC00152 silencing could be detected genome-wide including hypomethylation in SFRP2 and ALDH1A3 gene promoters.

Conclusion Our results suggest that LINC00152 IncRNA can contribute to CRC pathogenesis by facilitating cell proliferation through up-regulation of several oncogenes/metastatic genes in WNT, Notch and TP53 pathways and of cyclin D1 cell cycle progression gene, furthermore, by affecting the promoter methylation status of certain CRC associated genes.