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Tissue-based proteomics: insight into molecular mechanisms in cervical carcinogenesis

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Background

Tissue-based proteomics is an evolving tool used in cancer research to characterize the pathophysiology of disease. However, the proteome alterations involved in cervical carcinogenesis are not extensively studied. This study aims to elucidate the differentially expressed proteins and offer insights into the cellular processes and pathways involved in the development of cervical cancer.

Methodology

The pathological regions of interest in the cervical squamous epithelium were micro-dissected from formalin-fixed paraffin-embedded (FFPE) tissue sections of six normal cervix cases, five HPV-associated squamous intraepithelial lesion (SIL), and six squamous cell carcinomas (SCC). The samples were trypsin digestion and subjected to high throughput liquid chromatography-electrospray ionization-tandem mass spectrometry (LC/ESI-MS/MS) and trapped ion mobility time-of-flight-mass spectrometry (timsTOF-MS), followed by quantification with MaxQuant and Perseus software. Bioinformatics analyses were carried out using DAVID, ConsensusPathDB, and STRING.

Results and Discussion

We identified a total of 3597 proteins with 589, 550, and 1570 proteins unique to the normal cervix, SIL, and SCC groups, respectively, while 332 proteins were similar across all three groups. The predominant protein found was histone. Interestingly, the quantification results showed an upward trend for the up-regulated proteins and a downward trend for the down-regulated proteins in the progression from normal to SIL and SCC. The main molecular function was the binding process, and the top biological processes were chromatin silencing for SIL compared to the normal cervix and nucleosome assembly for the SCC compared to SIL group. The key pathways involved were viral carcinogenesis and necroptosis, reflecting their role in cell proliferation, migration, and metastasis.

Conclusion

The identification of proteins and their associated pathways provides a deeper understanding of the underlying molecular mechanisms involved in HPV-associated cervical cancer.

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during the analytical pipeline [3] that is important to identify challenges of systematic bias challenge in this study. The objective of this study was to evaluate the quality of NGS data sequence using FastQC in the early part of transcriptomic analysis pipelines of antiproliferative effects of dichloromethane CN fractions extracts on human breast cancer cell lines for biomarker discovery.

Methodology

Dichloromethane *CN* leaves extract with 50% inhibitory concentration (IC_{50}) value of 108µg/mL was exposed to the human breast cancer cells, MCF-7 for 72hrs chosen from a previous study. RNA was extracted from the treated and untreated cells for transcriptomic sequence, NGS technology using the Illumina HiSeq4000 platform. The sequencing output was the raw data that must be removed from the adapter sequences by the short read trimmed using FastQC (Babraham Bioinformatics, UK). This analysis uses the Linux operating system, Ubuntu 18.0v and the adapter was removed by a barcode tool, FlexbarFlexbarth its command line and script. All results were presented in multiQC which aggregated the results and generated a single HTML report with plots to visualize and compare various metrics between the samples involved.

Results and Discussion

The output of multiQC was filed from FastQC. In the summary report of FastQC status, important attention was made based on sequence quality and sequence length distribution. After RNA sequencing using the paired ends modules samples generated GC overall percentage of 54% and a length of 150bp showing no difference in GC composition and no biased library complexity, differences in amplification, or library specific causes. The sequence quality resulted in a pass or good result in the green area and more than 30 phred scores for all samples. As a result, the total overrepresented sequences found in each library make up more than 0.1% of the total that was passed and good quality which does not affect the biological consideration. Overall, the quality of RNA-seq data was high and the sequence quality was also good.

Conclusion

Multi QC report using FastQC is an advantageous tool because it is relatively quick to generate and provides a clear method for comparing samples to determine consistency and identify problematic samples. From this study, the multiQC report contains high quality clean data that was downstream in all future transcriptomic analyses for biomarker discovery in breast cancer research.

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IGF-1 and IGFBP-1 expressions as the potential prognostic biomarkers in women with Endometrioid Endometrial Cancer (EEC)

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Background

Insulin-like growth factor-1 (IGF-1) and the IGF binding protein 1 (IGFBP-1) expressions have been shown to play a vital role in cancer biology, including endometrioid endometrial cancer (EEC). We examined the prognostic value of these locally expressed biomarkers in endometrial biopsies and correlated them with clinicopathological data of EEC.

Methodology

mRNA expression of *IGF-1* and *IGFBP-1* in the endometrial biopsies were analysed in patients with EEC (n=25) and control (n=25) cases using quantitative polymerase chain reaction (qPCR) method. These data were then validated using immunohistochemistry (IHC) analysis and combined with EEC cases form a separate cohort (n=71) comprised of consecutive patients who underwent hysterectomy at UKMMC, between the year of 2014 to 2019. Overall survival was evaluated using the Kaplan-Meier method, with differences compared using the log- rank test. Independent relationships between these biomarkers and clinicopathological data were assessed using multivariate logistic regression models.

Results and Discussion

The *IGF-1* and *IGFBP-1* mRNA expressions were not significantly different between both groups, EEC vs. control. However, IGF-1 expression in IHC analysis was observed to be highly expressed in the EEC compared to the control group, while IGFBP-1 had a low expression in the EEC cases ($P<0.05$). IGF-1 was significantly associated with prognostic features ofEEC ($P<0.05$), while no associations were found in the IGFBP-1 expression. In our sub-analysis, high IGF-1 and negative IGFBP-1 expression were significantly correlated with poor progression-free survival (PFS) in advanced stage of EEC ($P<0.05$). Univariate and multivariate analyses showed that IGF-1 served as a predictive biomarker in EEC survival. Therefore, we postulate the continuous high expression of local IGF-1 protein in EEC cells could lead to poor outcomes. Our findings support that the circulating estrogen and IGF-1 were independently associated with a higher risk of recurrence in patients with stage III and IV of EC (Merritt et al. 2021). We also propose that a shift in the local expression of IGFBP-1 and IGF-1 may serve as a biomarker for the prognosis of EEC development.

Conclusion

Local expression of IGF-1 and IGFBP-1 may serve as prognostic biomarkers for EEC.

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P-19

Pharmacological consequence of inhibiting MAPK p38 using Ralimetinib dimesylate on lipopolysaccharide-induced E-selectin and VCAM-1 expression in HUVEC

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Background

Endothelial dysfunction plays a prominent role in the pathogenesis of sepsis and is associated with life-threatening organ dysfunction. Lipopolysaccharide (LPS), a Gram-negative bacterial component, is an important sepsis-associated mediator that induces the expression of adhesion molecules such as E-selectin and VCAM-1 upon its binding to dedicated pattern recognition receptors on endothelial cells (EC) [1]. Endothelial E-selectin and VCAM-1 expression promotes leukocyte adhesion, and high leukocyte infiltration in various organs is associated with a poor prognosis in sepsis patients. As the expression of E-selectin and VCAM-1 is partly driven by the

activation of the MAPK p38 signaling pathway [2], it is unknown whether Ralimetinib dimesylate (RM), a selective p38 MAPK inhibitor, can be used as a treatment to reduce E-selectin and VCAM-1 expression once LPS-driven activation of EC has started. RM treatment was previously shown to reduce TNF-α production in LPS-induced macrophages *in vitro* [3]. In this study, I investigated the pharmacological effect of RM treatment on E-selectin and VCAM-1 expression in LPS-stimulated HUVEC was investigated.

Methodology

Ten μM of RM was added into the HUVEC medium at 0.5, 1, 1.5, and 2 hours after HUVEC was exposed to 1 μg/ml of LPS. After 2, 4, 5, and 6 hours of LPS exposure, the cells were trypsinized and subjected to flow cytometry analyses. The Mean Fluorescence Intensity (MFI) of E-selectin and VCAM-1- conjugated fluorochromes were determined in the treatment groups and statistically compared to LPS-stimulated HUVEC controls using one-way ANOVA and Bonferroni post-hoc test. Each group was represented by three biological replicates. The results were reported as mean + SD. Viability of HUVEC was monitored microscopically.

Results and Discussion

LPS induced the expression of E-selectin and VCAM-1 in HUVEC in a time-dependent manner. E-selectin and VCAM-1 were maximally expressed on HUVEC at 4 and 6 hours after LPS exposure, respectively. E-selectin expression was attenuated throughout the 6 hours' duration of LPS exposure upon post-LPS treatment with RM at 0.5, 1, 1.5, and 2 hours after LPS exposure. VCAM-1 expression was not affected upon post-LPS treatments with RM. These findings suggest that MAPK p38 to be an important signaling cascade mediating LPS-induced E-selectin expression and can be pharmacologically targeted to attenuate E-selectin, but not VCAM-1 expression.

Conclusion

RM can be used to pharmacologically target the p38 MAPK signaling pathway to attenuate the expression of E-selectin, but not VCAM-1, in LPS-activated EC. Follow-up *in vivo* study should be done to investigate the effect of RM on the expression of E-selectin and VCAM-1 in animal model of experimental sepsis.

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AST, ALT, Bilirubin and AST/ALT Ratio Role; COVID-19 Patients

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Background

Impaired liver function upon admission has been linked to the severity of COVID-19 infection, yet the data is debated [1]. Therefore, this retrospective study aimed to evaluate the liver function among COVID-19 patients during hospitalization and its association with the disease severity.

Methodology

Patients aged 18 to 80 years with positive COVID-19 at Hospital Raja Perempuan Zainab II (HRPZ II), Kota Bharu, Kelantan, with available AST, ALT, Bilirubin, and AST/ALT ratio data on admission, were retrospectively evaluated from March 2021 until March 2022. Disease severity was categorized based on the Annex 2e guidelines by Ministry of Health Malaysia, which further classified them into mild to moderate disease (Stage 1-3) and severe to critical illness (Stage 4-5). The AST, ALT, Bilirubin, and AST/ALT ratio levels on Day 1 admission were archived from the electronic medical record system and compared between the two groups. Statistical analysis was performed using SPSS version 27. This study was approved by (JEPeM-USM) with protocol code USM/JEPeM/21100691 and the Ministry of Health Malaysia NMRR-21-762-58458 (IIR).

Results and Discussion

The study involved a total of 168 COVID-19 patients with a mean (SD) age of 46.67(16.10) for mild to moderate and 56.66(12.41) for severe to critical. There was a significant age group for both groups (p-value=0.002). During hospitalization, 16(14.41%) patients progressed to death from severe to critically ill patients. Upon admission, the median (IQR) of AST and ALT were significantly higher in the severe to critical group compared to the mild to moderate group, [AST; 39.0(49.0) and 24.0(14.0), ALT 38.0(43.0) and 21.0(18.0)], $p<0.05$. However, there were no significant differences between both groups for bilirubin level and AST/ALT ratio. Non-survivors had higher AST and ALT levels compared to survivors, with median (IQR) of [AST 98.0(88.0) and 32.0 (26.0), ALT of 67.5(90.0) and 28.0(31.0), ($p<0.05$). Similarly, there were no significant differences between non-survivors and survivors for bilirubin and AST/ALT ratio. Our study supports the idea that abnormal liver function at admission has been shown to be associated with the disease severity and mortality of COVID-19 infection. Therefore, there is a need to observe hepatobiliary sequelae in COVID-19 survivors as there are dynamic changes in liver function following hospital discharge.

Conclusion

Abnormal AST and ALT level at admission has been shown to be associated with the disease severity and mortality of COVID-19 infection. Further study needed to evaluate liver damage in COVID-19 post-discharge.

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MALDI-TOF mass spectrometry-based strategy in discovery reproducible *N*-glycans in human fibroblast

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