BMC Proceedings 2022, **16**(Suppl 7):7
https://doi.org/10.1186/s12919-022-00237-8

BMC Proceedings

MEETING ABSTRACTS Open Access

Conference Proceedings – 6th International Conference on Molecular Diagnostics and Biomarker Discovery (MDBD 2022): Building Resilience in Biomedical Research

Penang, Malaysia. 11-13 October 2022

Published: 21 December 2022

O-1

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

Gaayathri Kumarasamy[1], Mohd Nazri Ismail[1, 2], Sharifah Emilia Tuan Sharif[3], Christopher Desire[4], Parul Mittal[4], Peter Hoffmann[4] and Gurjeet Kaur[1]
[1]Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, 11800 Minden, Pulau Pinang, Malaysia; [2]Analytical Biochemistry Research Centre (ABrC), Universiti Sains Malaysia, 11900 Bayan Lepas, Pulau Pinang, Malaysia; [3]Department of Pathology, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia; [4]Clinical Health Sciences, University of South Australia, City West Campus, Adelaide, South Australia, 5000, Australia
Correspondence: Gurjeet Kaur (gurjeet@usm.my)
BMC Proceedings 2022, **16(Suppl 7):**O-1

From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

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.

Acknowledgement: The work was funded by the Fundamental Research Grant Scheme (FRGS/1/2019/SKK13/USM/01/1) from the Ministry of Education, Malaysia.

References

[1] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA. Cancer J. Clin. 68 (2018) 394–424.
[2] W.S. Jr, M.A. Bacon, A. Bajaj, L.T. Chuang, Cervical cancer: a global health crisis, (2017) 1–9.
[3] M. Vu, J. Yu, O.A. Awolude, L. Chuang, Cervical cancer worldwide, Curr. Probl. Cancer. 42 (2018) 457–465.

P-11

Determining an optimal DNA isolation method for rodent fecal samples by 16S rDNA bacterial diversity identification

Nik Amirah Auni Binti Nik Mohd Asri, Zarina Amin and Yew Chee Wei
Biotechnology Research Institute, Universiti Malaysia Sabah, Sabah, Malaysia
Correspondence: Nik Amirah Auni Binti Nik Mohd Asri (nikamirahauni@gmail.com)
BMC Proceedings 2022, **16(Suppl 7):**P-11

From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background
The choice of an optimum DNA isolation method from fecal samples is a constant challenge as ineffective lyses of diverse bacterial cells may lead to bias detection in the representation of a bacterial community in a sample. In addition, degraded DNA and presence of PCR inhibitors such as humic acid and polysaccharides carried over from fecal samples have been known to further reduce PCR efficiency. The purpose of this study is to determine an optimal DNA extraction method for rodent samples by assessing the general bacterial diversity based on PCR products amplified from the partial 16S rDNA gene. The PCR-DGGE technique separates the identical size of PCR amplicons by their differential mobility on gel based on sequence variations.
Methodology
In this study, five (5) fecal samples from wild rodents were collected and added into the Zymo Research DNA/RNA Shield reagent as preservative. Six DNA extraction methods i.e. QIAamp PowerFecal Pro DNA Kit (QIAGEN), the QIAamp AllPrep PowerViral DNA/RNA Kit (QIAGEN), the QIAamp UCP Pathogen Kit (QIAGEN), the ZymoBIOMICS DNA Miniprep Kit (ZYMO Research), and two conventional methods using different component in lysis buffer which were guanidium thiocyanate and CTAB were tested using between 10 to 15 mg of feces. The samples were extracted according to manufacturer's protocol and previous article [1] with some modifications, respectively. The extracted DNA were then subjected to amplification of V2 to V3 region of the bacterial 16S rDNA gene and DGGE separation of the amplicons. The DGGE banding pattern was analysed using the GelCompar II software (Applied Maths, Belgium) to compare the microbial diversity by using the Shannon-Weaver index [2].
Results and Discussion
The results showed that DNA yield varied with the extraction method; where the conventional method using guanidium thiocyanate showed a higher yield (average 324.22 ng/ul) than the other methods (average 45.33 ng/ul for the QIAamp PowerFecal Pro DNA Kit, 51.92 ng/ul for the QIAamp AllPrep PowerViral DNA/RNA Kit, 220.74 ng/ul for the QIAamp UCP Pathogen Kit, 42.4 ng/ul for ZymoBIOMICS DNA Miniprep Kit, and 165.74 ng/ul for the conventional method using CTAB). Majority of DNA extracted showed degradation when checked by gel electrophoresis. The QIAamp PowerFecal Pro DNA Kit was observed to show intact DNA. The DGGE profiles showed that the QIAamp PowerFecal Pro DNA Kit and QIAamp AllPrep PowerViral DNA/RNA Kit extracted the highest number of DNA bands (total of 168 and 167), while QIAamp UCP Pathogen Kit, ZymoBIOMICS DNA Miniprep Kit, and both conventional methods using guanidium thiocyanate and CTAB recorded 161, 160, 147, and 152 total of bands, respectively.
Conclusion
As a conclusion, QIAamp PowerFecal Pro DNA kit is the optimum DNA isolation method for rodent fecal samples as it provides better quality of DNA and microbial diversity in their DGGE profiles. Furthermore, this method is much time efficient QIAamp PowerFecal Pro DNA Kit and QIAamp AllPrep PowerViral DNA/RNA Kit produced more bands on their DGGE profiles than the other methos due to their use of bead-containing lysing matrix and vigorous homogenization.

References
[1] Chomczynski P, et al. The single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction: twenty-something years on. Nature Protocols, 2006, 1:2:581-585.
[2] Boon N, et al. Evaluation of nested PCR-DGGE (denaturing gradient gel electrophoresis) with group-specific 16S rRNA primers for the analysis of bacterial communities from different wastewater treatment plants. FEMS Microbiology Ecology, 2002, 39:2:101-112.

P-12

The toxic effects of *p*-Cresyl Sulfate on bone metabolism

Ahmad Faizal Bin Jelani[1], Gabrielle Ruth Anisah Fromming[2], Dayang Erna Zulaikha Binti Awang Hamsin[3] and Mohammad Zulkarnaen Bin Ahmad Narihan[1]
[1]Department of Pathology, Faculty of Medicine and Health Sciences, Sarawak, Malaysia; [2]Department of Basic Medical Sciences, Faculty of Medicine and Health Sciences, Sarawak, Malaysia; [3]Department of Paraclinical Sciences, Faculty of Medicine and Health Sciences, Sarawak, Malaysia
Correspondence: Mohammad Zulkarnaen Bin Ahmad Narihan (anmzulkarnaen@unimas.my)
BMC Proceedings 2022, **16(Suppl 7):**P-12

From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background
p-Cresyl Sulfate (*p*CS) is a uremic toxin that has been implicated in kidney disease, cardiovascular risks, endothelial dysfunction and neuropathies. In chronic kidney disease (CKD), *p*CS progressively accumulates in the body as the dysfunctional kidneys have a reduced ability to excrete toxins normally. *p*CS accumulated as a consequence of CKD cannot be removed from th body through dialysis, hence leading to further accumulation. This ultimately leads to bone loss which correlates with the worsening of CKD. As such, *p*CS could play a part in the development of bone loss with CKD. The objective of this review is to further understand the comprehensive effects pCS has on the bone.
Methodology
An extensive literature search was conducted in PubMed using the following keywords, '*p*-Cresyl Sulfate' OR '*p*-Cresol Sulfate' OR '*p*-Cresyl Sulphate', 'uremic toxin' OR 'uraemic toxin' AND 'bone' OR 'osteoblast' OR 'osteoclast' OR 'osteocyte'. From 2013 to 2022, 54 papers were found that contained the following keywords. Out of the 54 papers, 27 papers with significance were selected. The inclusion criteria for the study are in vivo and in vitro studies that examined the effects of *p*CS on bone. The exclusion criteria for the study is as follows: review article. After reviewing each article, 5 papers were selected for this review.
Results and Discussion
*p*CS is a prototype protein-bound uremic toxin associated with a multitude of toxic biological and biochemical effects. *p*CS is a substrate for human organic anion transporters (hOAT) 1 and 3 which is expressed in osteoblasts and other tissues. hOAT1 and hOAT3 may have a physiological role as large-capacity *p*CS transporters which could be a factor in the accumulation of *p*CS to toxic levels in osteoblasts [1]. Accumulation of *p*CS affects sclerostin production. As an inhibitor of the Wnt-signalling pathway, sclerostin prevents osteoblast formation and osteoprotegerin (OPG) production, inhibits osteoblast-mediated bone formation, and stimulates bone resorption by stimulating RANKL expression in osteocytes [2]. *p*CS also increase apoptosis and reduce osteoblast proliferation and viability with or without increased oxidative stress. This manifests by downregulation of the parathyroid hormone receptor (PTHR) on osteoblasts and a decrease in PTH-stimulated cAMP production through activation of c-Jun N-kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) [3]. and triggers JNK phosphorylation by attenuating PTH responsiveness in the bones under CKD conditions.

Conclusion
This review evaluates the toxic effects *p*CS induces on bone. However, further studies must be conducted to understand the mechanism of action and full effects *p*CS has on bone metabolism. In the future, *p*CS could potentially become a new therapeutic target for the management of bone disorders.

Acknowledgment: The authors acknowledged the financial support from the Ministrv of Higher Education Malavsia through Fundamental Research Grant Scheme (FRGS) with grant number of FRGS/1/2021/SKKO/UNIMAS/01/2

References
[1] Watanabe, Hiroshi et al. "Human organic anion transporters function as a high-capacity transporter for p-cresyl sulfate, a uremic toxin." *Clinical and experimental nephrology*vol. 18,5 (2014): 814-20. doi:10.1007/s10157-013-0902-9
[2] Desjardins, Lucie et al. "Uremic toxicity and sclerostin in chronic kidney disease patients." *Nephrologie & therapeutique* vol. 10,6 (2014): 463-70. doi:10.1016/j.nephro.2014.04.002
[3] Tanaka, Hisae et al. "p-Cresyl sulfate induces osteoblast dysfunction through activating JNK and p38 MAPK pathways." *Bone* vol. 56,2 (2013): 347-54. doi:10.1016/j.bone.2013.07.002

P-13

Characterization of ssDNA aptamers against ACE2 protein as therapeutic targets for COVID-19 infection

Zi Yuan Chang[1], Falah Abbas Muhamad Salih[1, 2] and Kai Ling Chin[1]
[1]Department of Biomedical Sciences, Universiti Malaysia Sabah, Sabah, Malaysia; [2]Department of Medical Laboratory Science, Cihan University, Erbil, Iraq
Correspondence: Kai Ling Chin (chinkl@ums.edu.my)
BMC Proceedings 2022, **16(Suppl 7):**P-13

From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background
Coronavirus disease 2019 (COVID-19), a highly contagious and rapidly spreading disease with significant fatality in the elderly population has swept across the world for the past three years since 2019. The enormity scale of this pandemic has resulted in the emergence of several SARS-CoV-2 variants with Omicron, the current main circulating variant of concern [1]. Nonetheless, all the variants identified to date share the same cell entry mechanism where the process is initiated when the spike protein of the viruses attach to the angiotensin-converting enzyme 2 (ACE2) receptors on their host cells [2]. Therefore, blocking the spike protein-ACE2 interaction using a biological binder targeting the ACE2 is a viable strategy for COVID-19 treatment. Aptamer is a short length of nucleic acid [RNA or single-stranded DNA (ssDNA)] which is selected through an *in vitro* selection called systematic evolution of ligands by exponential enrichment (SELEX). The selected aptamers have a 3D conformation that can specifically bind to their target with high affinity and pose many superior properties such as being smaller in size, thermally stable, and nearly non-immunogenic.
Methodology
Single-stranded DNA library of 76-bp oligonucleotides containing a randomized core region of 40 nucleotides, flanked by primer binding regions of 18 nucleotides on each side (5'-ATACCAGCTTATTCAATT-N40-AGATAGTAAGTGCAATCT-3') was synthesized. Recombinant His-tagged ACE2 protein (BBI Life Sciences, China) was immobilized to Ni-NTA Magnetic Beads (Gold Biotechnology, USA). Aptamers which showed high affinity towards the ACE2 were then selected from the initial library using SELEX. The isolated aptamers were cloned using the PCR Cloning Kit (NEB, USA) and the plasmids were extracted using DNA-spin Plasmid DNA Extraction Kit (iNtRON Biotechnology, Korea). The plasmids were sequenced and the resulting aptamer sequences were subjected to the UNAFOLD web server (http://www.unafold.org/) for ssDNA secondary structure prediction, followed by RNA tertiary structure prediction using RNAComposer (https://rnacomposer.cs.put.poznan.pl/), and finally, converted to equivalent ssDNA tertiary structure using Discovery Studio Visualizer v3.5. The aptamer-ACE2 interaction was predicted using the HDOCK docking program (http://hdock.phys.hust.edu.cn/).
Results and Discussion
Aptamers specifically bound to ACE2 were isolated after 13 rounds of magnetic bead-based SELEX. Here, we reported one of the isolated aptamers, Apt15, which potentially binds to the ACE2. The UNAFOLD revealed that this aptamer has a hairpin loop structure and single-stranded region with a Gibbs free energy value of -0.10. The low free energy value indicates that the ssDNA structure is thermodynamically stable [3]. *In silico* 3D molecular docking demonstrated that the single-stranded region of Apt15 binds to the ACE2 at the site recognized by the SARS-CoV-2 spike protein with a confidence score of 0.9894, thus suggesting the potential application of this aptamer as a therapeutic target for COVID-19 infection.
Conclusion
In conclusion, one ssDNA aptamer targeting ACE2 with therapeutic potential against COVID-19 was successfully identified in this study. Further investigations are necessary to determine its binding affinity to ACE2 and its ability to block the virus spike protein-ACE2 interaction. In future, this aptamer could serve as a broad-spectrum inhibitor against any existing or future emerging viruses that also use ACE2 for cell entry.

Acknowledgement: The work was funded by the Skim Dana Khas (SDK0226-2020) from the Universiti Malaysia Sabah.

References
[1] Parums DV. World Health Organization (WHO) Variant of Concern Lineages Under Monitoring (VOC-LUM) in response to the global spread of lineages and sublineages of omicron, or B.1.1.529, SARS-CoV-2. Med Sci Monit. 2022, 28: e937676-1.
[2] Han P, et al. Receptor binding and complex structures of human ACE2 to spike RBD from omicron and delta SARS-CoV-2. Cell. 2022, 185:630-640.
[3] Navien TN, et al. In silico molecular docking in DNA aptamer development. Biochimie. 2021, 180: 54-67.

P-14

Detection of *Campylobacter jejuni* and *Campylobacter coli* from retails broiler chicken by duplex PCR

Susilahwati Muhammad, Zaidah Abdul Rahman and Nur Syafikah Muhammad Nasir
Department of Medical Microbiology & Parasitology, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian,16150 Kelantan, Malaysia
Correspondence: Zaidah Abdul Rahman (drzaidah@usm.my)
BMC Proceedings 2022, **16(Suppl 7):**P-14

From The International Conference on Molecular Diagnostics & Biomarker Discovery 2022 (MDBD 2022)
Penang, Malaysia. 11 – 13 October 2022

Background
Campylobacter is one of the leading causes of foodborne diarrhoea illness in the developed countries and cause a significant public health concern worldwide. This study was designed to determine the prevalence of *Campylobacter coli and C.jejuni* contamination in poultry retails meat in Kota Bharu by duplex PCR from direct samples.
Methodology
A total of 50 fresh and chilled poultry chicken meat were purchased from 13 retail markets in. The samples were put into polyethylene bag and wash with Phosphate-buffered solution, the bacterial lysate was prepared directly from chicken wash, enriched in CCDA broth for 48 hours and the presence of *C.coli* and *C.jejuni* were detected by duplex PCR.
Result and discussion
Overall results revealed 22 % contamination of *Campylobacter* occur in poultry retail meat in Kota Bharu, Kelantan. Out of the fifty