antioxidant and anti-inflammatory and potentially anticancer like activities (anti-apoptotic effect in health or injury cells). There are many factors and variables that contribute to the pathogenesis of cancers such as oxidative stress, DNA damage and mutation, ionising radiations, carcinogenic chemicals.

**Material and methods** H₂ has significant potential to reduce somatic mutation through the reduction of excessive reactive oxygen species (ROS).

Formation of ROS is strongly related to the emergence of several human pathologic conditions such as atherosclerosis, neurodegenerative diseases, and ageing as well as certain types of human cancers including lung, breast and colon.

ROS are generated in organisms by γ, X, and UV radiation, bio transformation of dietary chemicals and some diet components.

Somatic mutation is a genetic alteration acquired by a cell that can be passed to another mutated cell in the course of cell division.

Moreover, it has shown through medical studies that H₂ has a protective effect against chemotherapy drugs.

**Results and discussions** To illustrate that, it is recognised a potential effect of H₂ for improving the quality of life of patients during chemotherapy by efficiently mitigating the side effects of anticancer drugs by decreasing oxidative stress, ameliorating metamorphosis due to decreased apoptosis.

H₂ also exhibits radio-protection by protecting the immune system.

Furthermore, H₂ may alleviate the haematological injury induced by radiation through the suppression of radiation-induced caspase 3 activation, in addition to rescuing the radiation-induced depletion of white blood cells and platelets.

Although anticancer properties of H₂ have been suggested, the mechanism(s) and efficiency by which H₂ act at the cellular level remained to be established.

It has demonstrated recently that H₂ water enhances the cancer cell apoptotic effect of 5-FU.

**Conclusion** In this study, the obtained data suggest that hydrogen water increased the inhibitory effect of 5-FU on colon 26 cells, and enhance the anticancer activity of 5-FU both in vivo and in vitro and these effects of hydrogen water are related to the hydrogen levels. Hydrogen water administration improved the survival of mice with colon 26 induced cancer.

Therefore, seeking for molecular markers able to predict therapeutic response to retinoid administration is undoubtedly an important aspect of their use in clinical practice. Several putative biomarkers indicating sensitivity or resistance of NBL cells to retinoids were reported in recent studies. The main aim of our study was to analyse the expression of five candidate proteins (PBX1, HOXC9, HMGA1, HMGA2 and DDX39A) in one experimental cohort (NBL cell lines; relevant FFPE tumour samples).

**Material and methods** In this study, 20 patient-derived NBL cell lines were used for the experiments. Sensitivity or resistance to natural (all-trans retinoic acid, 13-cis retinoic acid, 9-cis retinoic acid) and synthetic (fenretinide, bexarotene) retinoids was determined by MTT assay. Endogenous expression of the candidate biomarkers was analysed both on mRNA (RT-PCR) and protein (immunoblotting) levels in cell lines and on protein level (immunohistochemistry) in FFPE tumour samples. Changes in expression of these markers after treatment with retinoids were also analysed.

**Results and discussions** NBL cell lines resistant to retinoids showed either presence of HMGA2 or increased expression of HMGA1 together with PBX1. Cell lines without a detectable expression of HOXC9 is on both mRNA and protein level are resistant to retinoids. Increase of expression of HOXC9 protein after retinoid treatment was detected in sensitive cell lines only. Very strong expression of PBX1 protein was found in tumour samples taken from patients showing resistance or poor clinical outcome after treatment with retinoids.

**Conclusion** Our experimental study confirmed the usefulness of selected putative markers indicating sensitivity or resistance of NBL cells to retinoids in one experimental cohort consisting of patient-derived cell lines and respective tumour samples.

This study was supported by the project AZV MZCR 15-34621A.

**PO-215**

**RESISTANCE TO RETINOIDS – ANALYSIS OF PUTATIVE BIOMARKERS IN NEUROBLASTOMA CELLS AND TUMOUR TISSUE SAMPLES**

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**Introduction** Retinoids represent a popular group of differentiation inducers that are successfully used in oncology for treatment of neuroblastoma (NBL) in children. However, differentiation therapy has some limitations including toxicity and intrinsic or acquired resistance to retinoids observed in many patients.

Therefore, seeking for molecular markers able to predict therapeutic response to retinoid administration is undoubtedly an important aspect of their use in clinical practice. Several putative biomarkers indicating sensitivity or resistance of NBL cells to retinoids were reported in recent studies. The main aim of our study was to analyse the expression of five candidate proteins (PBX1, HOXC9, HMGA1, HMGA2 and DDX39A) in one experimental cohort (NBL cell lines; relevant FFPE tumour samples).

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**PO-216**

**METABOLIC AND MOLECULAR PROGRAMMING INDUCED DUE TO HYPERGLYCEMIA IN BREAST CANCER**

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**Introduction** Cancer and diabetes are the leading cause of mortality worldwide. Recent literature suggests alarming association between hyperglycemia and various cancers. But the complications induced due to hyperglycemia associated risk factors in breast cancer are not well established. In this study, an in depth analysis is done to understand the metabolic and molecular programming induced due to hyperglycemia in breast cancer.

**Material and methods** The effect of hyperglycemia in breast cancer was studied via various techniques. Proliferation, long term survival and molecular events were analysed using doubling time, colony formation, microarray, immunoblotting and immunofluorescence assays. Cell cycle analysis was performed via FACS PI staining. TRANSFAC approach was employed for gene sequence analysis. Pharmacological and siRNA mediated knockdown studies were used for studying targeted molecular events.

**Results and discussions** In silico screening for molecules with TFBS on cell-cycle regulating agents which were enhanced due to hyperglycemia in breast cancer cells was carried out via
TUMOUR SUPPRESSOR FUNCTION OF THE MICRORNA IN MITOCHONDRIA OF MULTIDRUG RESISTANT CELLS

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tumour cells. Our findings demonstrate that interference with cytoplasmic signalling pathways regulating p66Shc activation may be therapeutically exploited to alter ROS levels in tumour cells.

Conclusion

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Introduction

Mitochondria consist of a unique genome which can encode proteins involved in the electron transport chain (ETC) for energy production. Mitochondrial dysfunction can lead to cancer formation.

MicroRNAs (miRNAs) are evolutionary conserved, non-coding RNA molecules. MicroRNAs together with Argonaute (Ago) protein are essential for post-transcriptional gene regulation. Growing evidence suggested that nuclear-encoded miRNAs are imported to mitochondria. Despite their existence, their biogenesis and action mechanisms remain unexplored.

This project aims at investigating the roles of miRNAs in mitochondria. In particular, we are going to examine whether miRNAs can regulate oxidative phosphorylation (OXPHOS) and their contribution to the development of multidrug resistance (MDR) in hepatocellular carcinoma (HCC).

Material and methods

Accumulation of doxorubicin (DOX) in cells after co-treatment with ETC inhibitor for 4 hours was examined by flow cytometer. Mitochondria were purified by Western blotting and real time-PCR analysis respectively.

Results and discussions

Doxorubicin resistant HepG2 (R-HepG2) cell line has been developed in our lab. P-glycoprotein and Bcl-2, which are vital for MDR are highly upregulated in R-HepG2 cells compared to HepG2 cells. We confirmed that R-HepG2 cells produced more ATP than HepG2 cells. The amount of DOX retained inside R-HepG2 cells increased significantly after compromising the efficiency of OXPHOS by ETC inhibitors treatment. We believe that OXPHOS plays an essential role in maintaining MDR.

Subsequently, we identified several miRNAs that had differential expression level inside the mitochondria of HepG2 and R-HepG2 cells. Besides, Ago 2 protein, which is the key component in mediating RNA silencing, can also be detected in isolated mitochondria.

Conclusion

The current effort has preliminarily demonstrated OXPHOS are responsible for MDR in HCC. Moreover, Ago 2 protein and miRNAs can be found in mitochondria of MDR cancer cells. Further investigation on the relation between miRNAs inside mitochondria and mitochondrial-encoded proteins may shed light on their function in controlling OXPHOS.

MicroRNA In Mitochondria of Multidrug Resistant Hepatocellular Carcinoma

SF Lam*, SK Kong. The Chinese University of Hong Kong, Biochemistry, Hong Kong, China

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Introduction

Elevated levels of reactive oxygen species (ROS) frequently observed in tumours are part of the metabolic reprogramming, which is critical for cancer initiation and progression. However, cancer cells remain sensitive to treatments which further increase or decrease ROS. Tailored modulation of ROS levels thus may become a new strategy in cancer therapy. In contrast to other tumours, melanoma have low ROS levels and we have shown previously that RAF kinases are able to prevent excessive mitochondrial ROS production. To understand the underlying reasons for impaired ROS production in BRAFV600E transformed cells, we studied a possible link to the oxidoreductase p66Shc, a protein that is directly involved in the generation of ROS by the electron transport chain (ETC) in mitochondria. Despite their existence, their biogenesis and action mechanisms remain unexplored.

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