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August 5-6, 2019 | Singapore

Video Presentation

30 YEARS OF EXCELLENCE IN MEDICAL PUBLISHING

Intervention of lipid mediator based pathway for skin cancer

Ravi P Sahu
Wright State University, USA

The occurrence of skin cancers, particularly, malignant melanoma and its associated mortality rates have risen globally over the last several decades. Among various risk factors, exposure to reactive oxygen species (ROS)-generating stimuli have been implicated in several pathophysiological processes including skin cancer. While several immune and non-immune mediated mechanisms have been proposed for skin cancer, the molecular events governing the initiation and/or progression of melanoma or non-melanoma skin cancers are yet to be fully explored, given the diverse nature of ROS-generating stimuli. Studies, including ours, have implicated the critical roles of a potent lipid mediator, Platelet-activating factor (PAF) in augmenting the growth of melanoma and non-melanoma skin cancers in various experimental models. Importantly, accumulating evidences indicate that PAF and PAF-like ligands can be produced via several ROS-generating stimuli including ultraviolet B (UVB), cigarette smoke, jet fuel, tumor promoters and therapeutic agents. Notably, our studies have demonstrated that PAF/PAF ligands produced via many of such stimuli induce systemic immune suppression in a PAF-receptor (PAF-R) dependent manner. This systemic immune suppression resulted in enhanced growth of experimental skin tumor types via mechanisms mediated by PAF-R-dependent upregulation of cyclooxygenase type 2 (COX-2), related ecosanoids and immunophenotypes such as regulatory T cells (Tregs) and cytokines including interleukin 10 (IL-10). Of importance, ours and other groups have shown that therapeutic agents (i.e. chemotherapy and radiation therapy)-generated PAF ligands impede their efficacy in experimental melanoma models, in a process pharmacologically blocked by agents including PAF- R antagonists, COX-2 inhibitors and depleting antibodies against Tregs and IL-10. Of significance, we identified increased PAF or PAF-R activity in tumor samples/perfusates collected from melanoma and non-melanoma patients, who underwent scheduled treatments with chemotherapy or radiation therapy. These findings indicate that targeting PAF-R-mediated pathway could be explored as a promising appraoch for the intervention of skin cancer.

Biography

Ravi P Sahu has completed his PhD from Sanjay Gandhi Post Graduate Institute of Medical Sciences and postdoctoral studies from the University of Pittsburgh Medical Center, Texas Tech University Health Science Center and Indiana University School of Medicine. He is currently an Assistant Professor at the Department of Pharmacology and Toxicology at Wright State University Boonshoft School of Medicine at Dayton, OH. His laboratory has been interested in defining the role and mechanisms of a potent lipid mediator, platelet-activating factor (PAF)-mediated pathway in cancer growth and efficacy of cancer therapies using various *in-vitro* and *in-vivo* experimental model systems as well as human samples. The overall goal is to delineate novel approaches based on this PAF pathway for the intervention of melanoma and non-melanoma cancers. He has published over 50 papers in reputed journals and has been serving as an editorial board member and adhoc reviewer of several journals.

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On the performance of variable selection and classification via ranked based classifier

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In high-dimensional gene expression data analysis, the accuracy and reliability of cancer classification and selection of important genes play a very crucial role. To identify these important genes and predict future outcomes (tumor vs. non-tumor), various methods have been proposed in the literature. But only few of them take into account correlation patterns and grouping effects among the genes. In this article, we propose a rank-based modification of the popular penalized logistic regression procedure based on a combination of l1 and l2 penalties capable of handling possible correlation among genes in different groups. While the l1 penalty maintains sparsity, the l2 penalty induces smoothness based on the information from the Laplacian matrix, which represents the correlation pattern among genes. We combined logistic regression with the BH-FDR (Benjamini and Hochberg false discovery rate) screening procedure and a newly developed rank-based selection method to come up with an optimal model retaining the important genes. Through simulation studies and real-world application to high-dimensional colon cancer gene expression data, we demonstrated that the proposed rank-based method outperforms such currently popular methods as lasso, adaptive lasso and elastic net when applied both to gene selection and classification.

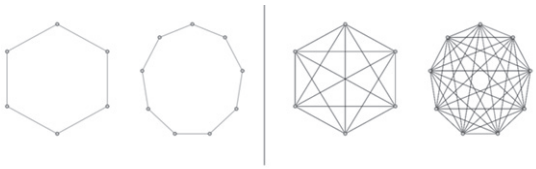

Figure 1. The ring network (left) and F.con network (right) are shown for the case there are two genes consisting of 6 and 9 CpG sites, respectively.

Table 9. List of top 5 ranked genes across rank-based, lasso, adaptive and elastic net. An extra asterix (*) sign is put next to a gene each time the gene is selected by one of four methods.

EST Name	Gene ID	Gene Description	Selection Probability
Rank-Based			
***Hsa.36689	Z50753	H.sapiens mRNA for GCAP-II/uroguanylin precursor	1.00
***Hsa.692.2	M76378	Human cysteine-rich protein (CRP) gene, exons 5 and 6	0.99
**Hsa.37937	R87126	Myosin heavy chain,nonmuscle(Gallus gallus)	0.97
****Hsa.1660	H55916	Peptidyl-prolyl cis-trans isomerase, mitrochondrial precursor(human)	0.91
Hsa.1832	R44887	nedd5 protein (Mus musculus)	0.90
Lasso			
***Hsa.36689	Z50753	H.sapiens mRNA for GCAP-II/uroguanylin precursor	0.87
Hsa.692.2	M76378	Human cysteine-rich protein (CRP) gene, exons 5 and 6	0.82
*****Hsa.1660	H55916	Peptidyl-prolyl cis-trans isomerase, mitrochondrial precursor(human)	0.66
Hsa.6814	H08393	Collagen alpha 2(XI) chain(Homo sapiens)	0.52
Hsa.8147	M63391	Human desmin gene, complete cds	0.50
Adaptive Lasso			
Hsa.1454	M82919	H. gamma amino butyric acid(GABAA)receptor beta3 subunit mRNA,cds	0.83
Hsa.6814	H08393	Collagen alpha 2(XI) chain(Homo sapiens)	0.77
****Hsa.1660	H55916	Peptidyl-prolyl cis-trans isomerase, mitrochondrial precursor(human)	0.77
Hsa.14069	T67077	Sodium/Potasssium-transporting atpase gamma chain(Ovis aries)	0.69
Hsa.2456	U25138	Human MaxiK potassium channel beta subunit mRNA, complete cds	0.55
Elastic Net			
***Hsa.36689	Z50753	H.sapiens mRNA for GCAP-II/uroguanylin precursor	0.98
**Hsa.37937	R87126	Myosin heavy chain,nonmuscle(Gallus gallus)	0.94
***Hsa.692.2	M76378	Human cysteine-rich protein (CRP) gene, exons 5 and 6	0.94
Hsa.8147	M63391	Human desmin gene, complete cds	0.91
****Hsa.1660	H55916	Peptidyl-prolyl cis-trans isomerase, mitrochondrial precursor(human)	0.84

Biography

Showaib Rahman Sarker is pursuing his master's degree in Statistics at The University of Texas at El Paso. He has expertise in Statistical Machine learning application in High-Dimensional Gene Expression Data. Currently, he is doing his research in High throughput cancer gene expression data. His main goal is to find out the important genes which are responsible for cancer and classify (tumor vs non-tumor) accurately. He is passionate to apply statistical approach and machine learning approach in cancer research.

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Poster

30 YEARS OF EXCELLENCE IN MEDICAL PUBLISHING

Primary cardiac rhabdomyosarcoma successfully treated with eribulin: A case report

Taizo Hirata
Kure Medical Center and Chugoku Cancer Center, Japan

Background: Rhabdomyosarcoma (RMS) is the most common of soft tissue sarcomas (STSs) that occur commonly in children and adolescents. RMS is rare in adults. Furthermore, since cardiac tumors are also a rare disease, adult cardiac RMS is extremely rare. We herein report a case of cardiac RMS successfully treated with eribulin.

Case presentation: A 68-year-old female was admitted with a sudden loss of conscious. The cause of syncope was found to be due to a cardiac tumor, and the tumor was resected by emergency surgery that was diagnosed as embryonal rhabdomyosarcoma (eRMS). In the present case, although surgical treatment alleviated her symptoms, the residual tumor increased after surgery and needed multimodality treatment. First line chemotherapy with a VAC (vincristine, actinomycin D and cyclophosphamide) regimen was difficult to continue due to adverse events, and thus eribulin was used as a second line. Eribulin was considered as being more tolerable with less toxicity and maintained a stable disease (SD) status for more than 18 months.

Conclusion: To the best of our knowledge, this is the first reported case of cardiac RMS successfully treated with eribulin over a relatively long period. Our case suggests that eribulin therapy could be a treatment option for RMS.

Biography

Taizo Hirata is working in the Department of Medical Oncology in National Hospital Organization, Kure Medical Center and Chugoku Cancer Center at Kure-City, Hiroshima, Japan. His area of research includes breast cancer, oncology and drugs, cardiac rhabdomyosarcoma, Oncology pharmaceutical safety and Chemotherapy.

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Noninvasive methods of tumor detection and activity monitoring

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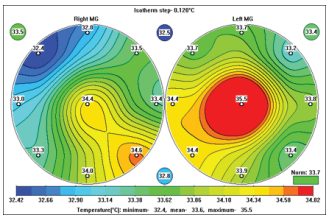
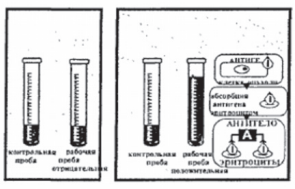
[2]Sklifosovsky Institute of Emergency Medicine, Russia



The RTM device measures temperature in a tiny amount of tissue (approx. 1 cm3) inside a body at depths up to 10 cm, with a precision of about 0.050C. A non-invasive detector of the device measures tissue's radiating heat within a spectrum of radio waves. The method was licensed by the Ministry of Healthcare in 1997 for use in early diagnosing of breast cancer, as well as for monitoring of this tumor during treatment courses. Over observations confirmed that conclusions are true for most of locations of cancer processes. Certain exceptions may only arise in cases of tumors in: kidneys, stomach, pancreas, and liver. Due to a very high rate of thermo-production in these organs areas of tumor heat may stay partially masked.



Ro-test. Immunology test was developed in P.A. Hertsen Moscow Oncology Research Center in 1995. This test indicates products of metabolism of embryonic genes on which wake up for activity while canceration. Numerous tests undertaken with a help of about 100,000 patients suffering from different kinds of cancer revealed that Ro-test gives up to 95% opportunity to reveal the existence of cancer of different locations and origins. It occurs at times that the test reveals false positive results, when the anti-A-serum accelerates sedimentation of red blood cells by more than 1.5 times (brink level for positive canceration). Comparative later research together with the RTM test results, that we have been collecting here in our Research Center for a couple of years, displays that in almost all these cases patients experienced a pre-cancer state at least in one of the inner organs.

The combined approach of both techniques arms physicians with a perfect ongoing real-time tracking of the treatment process and with means to define a stage of minimal metastasis risks, as well as to discover presence or absence of oncologic changes after the surgery.

Biography

Dmitry Malenkov is now studying Public health management and Economics in Higher School of Economics. Surgeon, cardiothoracic surgeon, oncologist, scientist, public health administrator, co-founder of Scientific center for integrative medicine. Dmitry Malenkov is the author of more than 40 papers, in the field of clinical and experimental medicine

Andrey Malenkov spent over 50 years of his life to studying the mechanisms of tumor appearance and growth. He worked in laboratory on cancerogenesis of Russian National Center of Oncology. Andrey Malenkov is the author of more than 80 papers, 20 books, a scientific discovery and several patents in the field of oncology and natural sciences. Over 20 years Prof. Malenkov heads a medical center for integrative oncology.

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Successful delivery of docetaxel to rat brain using experimentally developed nanoliposome: A treatment strategy for brain tumor

Salman Mondal
Jadavpur University, India

In the field of neuroscience research, the treatment of brain cancer remains a challenge due to poor permeation of conventional chemotherapeutic drugs across the blood–brain barrier (BBB). Docetaxel (DTX) is found to be very effective against glioma cell *in vitro*. However, *in vivo* passage of DTX through BBB is extremely difficult due to the physicochemical and pharmacological characteristics of the drug. No existing formulation is successful in this aspect. Docetaxel is known to be effective against various tumors, including brain glioma. Nanoliposomal formulation is a promising novel strategy for site-specific drug delivery, without affecting normal tissues. In this study, effort was made to send DTX through blood–brain barrier (BBB) to brain to treat diseases such as solid tumor of brain (glioma) by developing DTX-loaded nanoliposomes. Primarilydrug-excipients interaction was evaluated by FTIR spectroscopy. The DTX-loaded nanoliposomes (L-DTX) were prepared by lipid layer hydration technique and characterized physicochemically. *In vitro* cellc viability assay and cellular uptake in C6 glioma cells was investigated. Further, *in vivo* plasma and brain pharmacokinetic study by LC-MS analysis was performed. FTIR data show that the selected drug and excipients were chemically compatible. The vesicle size was less than 50nm with smooth surface. Drug released slowly from L-DTX *in vitro* in a sustained manner. The pharmacokinetic data shows more extended action of DTX from L-DTX in experimental rats than the free-drug and Taxotere. DTX from L-DTX enhanced 100% drug concentration in brain as compared with Taxoterein 4 h. Thus, nanoliposomes as vehicle may be an encouraging strategy to treat glioma with DTX.

Biography

Salman Mondal has completed his masters in pharmacy (clinical pharmacy and pharmacy practice) from Department of Pharmaceutical Technology, Jadavpur University, Kolkata, West Bengal, India. His project work on " Prospective observational study to asses patients profile with clostridium defficile colitis in ICU " and project has been done with collaboration of AMRI Hospital, Kolkata, West Bengal, India.

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Tamoxifen citrate loaded polymeric nanoparticles for enhanced breast cancer therapy

Angana Mondal
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Various chemotherapeutic agents are used to treat the breast cancer. The existing anticancer agents do not greatly differentiate between the cancerous and normal cells, leading to systemic toxicity and adverse effects. Drug permeation into the cancer cells from the conventional formulation is very poor due to less distribution and quick elimination. The extensive distribution and rapid elimination from targeted organs result in a greater require¬ment of the drug by the tissue, which causes undesirable toxicity. Polymeric nanoparticles play an important role in delivering such kinds of chemotherapeutic agents in a controlled manner. Nanoparticles make it possible to deliver the desired concentration of drug in the specific site, thus minimizing the side effects and reducing the toxicity. A number of novel formulations with Tamoxifen citrate loaded polylactide-co-glycolide (PLGA) based nanoparticles (TNPs) were developed and characterized. Their uptakes in Michigan Cancer Foundation-7 (MCF-7) breast cancer cells were also investigated. Nanoparticles were prepared by a multiple emulsion solvent evaporation method. Drug-excipients interaction, surface morphology, zeta potential and size distribution, cellular uptake were carried out . No chemical interaction was observed between the drug and the selected excipients. TNPs had a smooth surface, and a nanosize range (250–380 nm) with a negative surface charge. Sustained drug release pattern of the nanoparticles were internalized well in the cytoplasm by the MCF-7 breast cancer cells on a concentration dependent manner. Drug loaded nanoparticles were found to be more cytotoxic than the free drug. TNPs (NP-4) showed the highest drug loading and were taken up well by the MCF-7 breast cancer cell line *in vitro*. Thus the formulation may be suitable for breast cancer treatment.

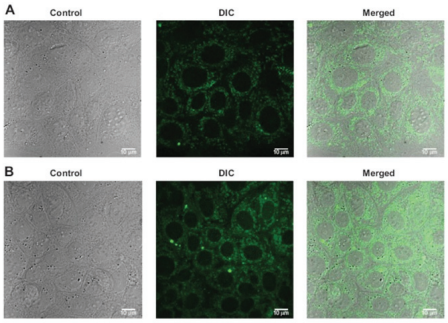

Figure 8 Confocal microscopy and differential interference contrast (DIC) images of MCF-7 cells.
Notes: (**A**) Formulation NP4 at concentration 50 µL for 3 hours; (**B**) Formulation NP4 at concentration 100 µL for 3 hours.

Biography

Angana Mondal has completed her masters in “Clinical Pharmacy & Pharmacy Practice” from Jadavpur University. Her thesis topic entitled “A Prospective Observational Study To Compare The Effects Of Ropivacaine With Bupivacaine in Brachial Plexus Block”. She has completed her thesis in collaboration with AMRI Hospitals, Kolkata, India.

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Immobilization of antitumor enzyme L-lysine alpha-oxidase from Trichoderma cf. aureoviride Rifai on the nanocomposite polyGraphene as matrix for cancer screening, diagnosis and treatment

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[2]Sklifosovsky Institute of Emergency Medicine, Russia

[3]Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russia

[4]Sklifosovsky Institute of Emergency Medicine, Russia

[5]RUDN University, Russia

It was discovered that expanded graphite – Nanocomposite of PolyGraphene (PG) obtained after hydro-termic treatment of modified graphite became to be able to interact as sorbent PG with enzyme L-lysine alpha-oxidase from Trichoderma cf. aureoviride Rifai.

It was studied the sorption properties of carbon material as an example of PolyGraphene (PG) concerning enzyme L-lysine alpha-oxidase from Trichoderma cf. aureoviride Rifai.

PG - version of ultrafine carbon sorbent, which was developed on the basis of the modified oxygen-containing expanded graphite (OCEG).

L-Lysine α-oxidase (LysOx) is one of the enzymes which are prospective in biotech-nology and medicine due to its antitumor and kinetic properties. An *in vivo* therapeutic effect was demonstrated on animals with tumor grafts: breast carcinoma SKBR3, Bro melanoma, intestinal cancer HCT116 and LS174T, ovary adenocarcinoma SCOV3, liver carcinoma.

This work aims to immobilize the extracellular L-lysine α-oxidase (LysOx) from Trichoderma cf. aureoviride Rifai VKM F-4268D on PolyGraphene and characterize some properties of adsorbed enzyme. Two types of PolyGraphene were used. Maximum adsorption equal to 5 or 11 μg protein/ mg of carrier was achieved, a high specific activity comparable to that of a free enzyme to take place.

LysOx adsorbed on PolyGraphene was shown to be a very stable system, namely high stability was revealed in the presence of chaotropic agent (urea) or proteolytic enzymes (pronase, chymotrypsin, trypsin).

Thus, the possibility of immobilization of LysOx on graphene with the full conservation of specific activity was shown.

In addition, LysOx is one of the enzymes that is promising in the enzyme therapy of tumors, based on the high sensitivity of tumor cells to the deficiency of growth factors, including amino acids.

Conclusions. Researchers have the theoretical and practical importance, the received results can be applied as matrix both in cancer screening, diagnosis and treatment and in cleaning of already known enzymes.

Biography

Dmitry Malenkov is now studying Public health management and Economics in Higher School of Economics. Surgeon, cardiothoracic surgeon, oncologist, scientist, public health administrator, co-founder of Scientific center for integrative medicine. Dmitry Malenkov is the author of more than 40 papers, in the field of clinical and experimental medicine.

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Design and development of apigenin loaded nanoparticles for the treatment of hepatocellular carcinoma in rats

Alankar Mukherjee
Jadavpur University, India

Hepatocellular carcinoma (HCC) is one of the most common malignant solid tumors with a very poor prognosis and survival rate in humans and HCC-related death has been reported as the second highest among the all cancer related deaths worldwide. Apigenin, a dietary flavonoid, possesses anti-tumor activity against HCC cells *in-vitro*. The apigenin loaded nanoparticles (ApNp) were developed. The physicochemical characterization of apigenin loaded nanoparticles (ApNp), biodistribution pattern and pharmacokinetic parameters of apigenin upon intravenous administration of ApNp, and effect of ApNp treatment in rats with HCC were investigated It was observed that Apigenin loaded nanoparticles had a sustained drug release pattern and it reached successfully to the hepatic cancer cells *in-vitro* as well as in liver of carcinogenic animals. ApNp predominantly delayed the progress of HCC in chemical induced hepatocarcinogenesis in rats. Quantification of apigenin was done by liquid chromatography–mass spectroscopy (LC-MS/MS) which showed that apigenin availability significantly increased in blood as well in the liver upon ApNp treatment. Thus, the severity of hepatocellular carcinoma was substantially controlled by Apigenin loaded and could be a future hope for lingering the survival in hepatic cancer patients.

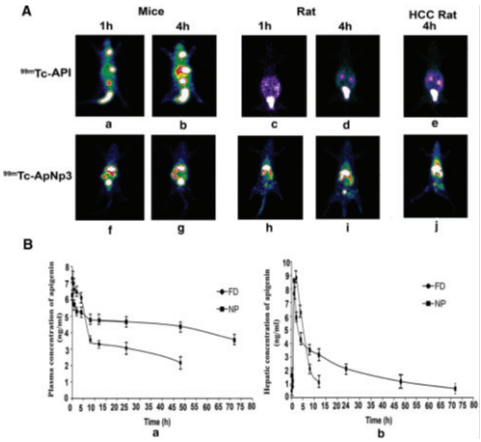

Figure . Pharmacokinetic data of apigenin from ApNp3//API and Gamma scintigraphic images of radiolabeled ApNp3/API and apigenin biodistribution in vivo (A). Pharmacokinetic profile of apigenin: plasma profile and hepatic accumulation of drug in experimental animal (B).(A) Time dependent biodistribution and accumulation of 99mTc-API in mice at 1 h (a) and 4 h (b); in rats at 1 h (c) and 4 h (d); in rats with HCC at 4 h (e) along with the accumulation of 99mTc-ApNp3 in mice at 1 h (f) and 4 h (g); in rats at 1 h (h) and 4 h (i); rats with HCC at 4 h (j).(B) Plasma (a) and hepatic (b) concentration of apigenin upon i.v. bolus injection (at a dose of 1 mg/kg body weight) of ApNp3 and API were shown.

Biography

Alankar Mukherjee has completed her M. Pharm in Clinical Pharmacy and Pharmacy Practice from Department of Pharmaceutical Technology, Jadavpur University, Kolkata, West Bengal, India. During her master's degree, she has worked on the project entitled "Pattern of use of various erythropoiesis stimulating agents in hemodialysis patients in a tertiary care hospital of Eastern India". The project has been done in collaboration with the AMRI hospital, Kolkata, West Bengal, India.

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Chitinase production from *Paenibacillus sp*. BISR-047 utilizing seafood waste as substrate under solid-state fermentation

Saavi Pradhan
Banasthali University, India

Chitinases have huge potential applications and biological value in industries; they are used in generating single cell proteins, sweeteners, insecticides, antifungal drugs, anti cancer agents, biopesticides, food processing agents, degrading agents for sea waste etc. Chitinases are used for the conversion of chitin a polysaccharide into monomers. Extraction of chitin involves two steps, demineralisation and deproteinisation, which can be conducted by two methods, chemical or biological. The chemical method requires the use of acids and bases, while the biological method involves microorganisms.

Solid-state fermentation (SSF) is a low-cost fermentation technology, particularly suitable for the needs of developing countries. This bioconversion technology of chitinous materials through chitinolytic process is an alternative waste treatment that not only solves environmental problems but also decreases the production costs of microbial chitinases. Therefore, efforts were made in the present study to utilize seafood waste for chitinase production under SSF. A novel thermo-tolerant bacterium Paenibacillus sp. BISR-047, previously isolated from the Great Indian Desert soils, was used and various process parameters were studied. We obtained 346 IU/ml of chitinase production in a medium containing crab and prawn waste (5:2; waste: water), 1.5 g/kg yeast extract (w/w), 0.5 g/kg NaCl (w/w), 40% moisture content, pH 8 and at 45 ºC temperature. We obtained 29% dry weight reduction after 10 d of incubation under SSF. Our results indicate scope for the utilization of seafood waste for industrial production of chitinase using SSF.

Biography

Saavi Pradhan has her expertise in the field of Microbiology and analytical research studies with experience contribution in microbiological studies of Great Indian Desert soils. Phytopathogens which cause severe damage to commercial crops were also studied and successful field trials have been done. Her work also involved pest control biological methodologies to overcome disease causing pest issues.

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Computational analysis of single nucleotide polymorphisms (SNPs) in human ATP1-A2 gene

Afra M Bkrye
University of Bahri, Sudan

BACKGROUND: In this study we analyzed the effect of genetic mutations mainly Single nucleotide polymorphisms (SNPs) mutations that can alter the expression and functions of ATP1A2 gene as gene had been candidate to cause Migraine.

METHODS: ATP1A2 gene was investigated in dbSNP/NCBI database in June 2016 and we used different computational analysis approach. Deleterious nsSNPs were predicted by SIFT and Polyphen-2 softwares. Then the damaging nsSNPs were submitted to I- mutant to Predict the change in stability due to mutation and PHD-SNP and SNPS&GO software used to demonstrate the relationship between SNP and related Migraine Protein structural analysis of amino acid variants was performed by Project Hope. To highlight genetic interactions of ATP1A2 we used Gene MANIA software.

RESULTS: Gena mania revealed that ATP1A2Gene encodes for the α2 subunit of ATPase Na+/ K. The SNPs sequence of ATP1A2 gene was collected from NCBI; 2576of them [Homo sapiens] only From sift and polyphen-2 software the high score deleterious SNPs were found as following: (rs28933401), (rs368405677), (rs121918612), (rs121918614), (rs121918615), (rs121918618), (rs121918619), (rs200425518), (rs181618883) and (rs149144720) Which analysis in Project HOPE software to analyzed the changing in amino acid properties and domains, which found among these (rs). (rs28933401), (rs368405677), (rs121918612) have 100% mutation. Additionally, I-Mutant and PHD-SNPs and SNP&GO showed decrease instability for these nsSNP sup on mutation. Protein structural analysis with these amino acid variants was performed by using I-Mutant, Swiss PDB viewer, to check their molecular dynamics and energy minimization calculations.

CONCLUSION: in this study we found R689Q, T378N, G301R, D718N, P979L, T415M, R171W, A688P, A297T and G855E mutations in ATP1A2 gene could directly or indirectly destabilize the amino acid interactions and hydrogen bonds networks thus explaining the functional deviations of protein To some extent mutations in ATP1A2 gene could directly or indirectly destabilize the amino acid interactions and hydrogen bonds networks thus explaining the functional deviations of protein to some extent.

Biography

Afra M Bkrye has completed MSC in Biotechnology at Sudan academy of science and technology. Currently she is working as lecturer at Bahri University, department of biochemistry and molecular biology.

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e-Poster

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Detection of mutations in hTERT gene promoter sequence without DNA amplification by novel SERRS sensor for cancer diagnostics

Zvereva M
Lomonosov MSU, Russia

Recently developed liquid biopsy analysis based on circulating tumor DNA (ctDNA) released by tumor cells in body liquids significantly enhanced non-invasive molecular diagnostics of cancer as ctDNA contains all mutations generated in the tumor. For example, mutations in the promoter of telomerase catalytic subunit (TERT) gene are highly specific for multiple cancers, including bladder cancer. Thus, selective determination of such mutations can improve early diagnostics of cancer and monitoring of cancer progression and treatment. Main limitations for existing methods of ctDNA detection in body fluids are: a) a low portion of tumor DNA in comparison to wild type circulating DNA that results in poor sensitivity, especially in case of preliminary DNA amplification and b) high fragmentation of cell free DNA that complicates DNA purification. In this regard, novel approaches without DNA isolation and amplification for specific detection of tumor DNA are of unmet medical need.

Spectroscopy Resonance Raman Scattering (SERRS) allows detection of target molecules in multicomponent complex mixtures without isolation at fM concentrations due to the resonant enhancement of the signal. To apply this approach, we developed SERS-active colloids based on silver nanoparticles deposited on planar sensor surfaces. Short oligonucleotide probes were immobilized on silver nanoparticles using anchor sequences. As a result, we were able to detect DNA with TERT promoter sequence from femtomolar to micromolar concentration with the linear response of SERRS signal.

The study was supported by Russian Foundation of Basic Research grant № 18-29-08040.

Biography

Zvereva M has her expertise in investigation of telomerase as molecular target for anticancer drug development and base of new molecular tests for diagnostics of oncogenic process. Her research group was concentrated on understanding of telomerase functioning and regulation using biochemical and bioengineering approaches for model organisms. They optimized the telomerase activity tests and used it in different ways (Biochemie, 2013; Mol Cell Biol., 2014; and etc.), created two novel classes of telomerase inhibitors for future anti-cancer therapy development (J Med Chem. 2014; Nucleic Acids Res., 2014) and one of them could be used possibly for diagnostics (Biochemistry (Moscow), 2015, review). The screening of telomerase activation in clinical samples and assessing the potential role of splicing events during activation of telomerase was done for cervical cancer and pre-cancerous lesion (Biochimie, 2010). As a scientist of IARC she participated in development of method for detection of mutations in hTERT gene promoter sequence for bladder cancer diagnostics (EBiomedicine, 2019).

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The role of a complex oral pathogen in oral cancer progression and prevention

Meena Vanamala
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Worldwide Oral Cancer incidence rates have consistently placed the disease among the top 10 most frequently occurring cancers. Amongst the range of etiological agents implicated in its initiation and progression, oral bacteria have only recently gained attention. The anaerobic Gram-negative bacterium *Fusobacterium nucleatum*, found in the mouth and GI tract is recognized as a mediator of periodontal disease, still births and a subset of colorectal cancers. In the intestine model of carcinogenesis, the distinctive adhesin FadA of *F. nucleatum* binds to E-Cadherin and induces pro-inflammatory and oncogenic protein pathways. Our study involved the investigation of cellular response of an oral dysplastic cell line, D20 to FadA and a related protein FadB. The relative changes in cellular signaling of four different markers NF-κB phosphor, C-myc, β-catenin and E-cadherin (known markers of inflammation, cell proliferation, oncogenesis, adhesion and invasion respectively) were measured using western blotting and the recently developed systems level proteomics method, Digiwest. Analysis of quantified data showed that FadA and FadB upregulate the same pathways in a mildly dysplastic oral cancer cell line as have previously been described for Colorectal cancer. Data from MTT and Trypan blue assays confirmed that these two proteins promote increased cellular proliferation of the D20. This study gave us new insights into the potential role of *F.nucleatum* FadA proteins in oral cancer, and in the process highlights the therapeutic possibilities of exploiting these molecules to design diagnostic screens, to develop them as targets for small molecule inhibitors and potentially to use them as vaccine candidates.

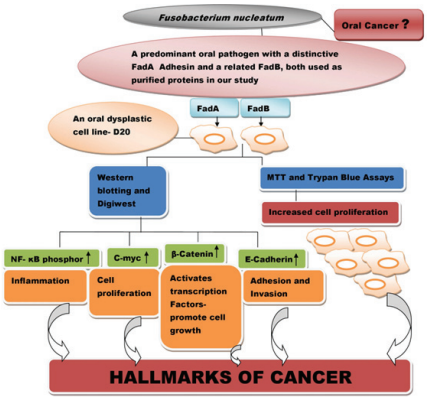

Figure1: Cellular responses of an oral dysplastic cell line to purified proteins FadA and FadB of Fusobacterium nucleatum. (: increase/ upregulation)

Biography

Meena Vanamala is a Dental Surgeon and a passionate young researcher. She received a Master of Research degree in Oral Cancer from the University of Dundee, United Kingdom. Her research on role of *Fusobacterium nucleatum* in oral cancer progression has garnered novel results and has extended the reach of this research to new horizons. She is currently working towards publishing her work in this subject. She plans to pursue a PhD and broaden her experience and perspective with a hope to contribute to a meaningful change. She loves reading and is fascinated by the cultural diversity across the globe.

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Investigating the nexus between DNA repair pathways and genomic instability in cancer

Sonali Bhattacharjee
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DNA double-strand breaks are one of the most lethal lesions to a cell that can be repaired by one of the two cellular pathways; non-homologous end joining or homologous recombination. Homologous recombination genes are particularly attractive targets for precision cancer therapy because these genes have altered expression patterns in cancer cells when compared with normal cells and these genetic abnormalities can be targeted for selectively killing cancer cells while leaving normal cells unscathed. Synthetic lethality is thought to be the new frontier of cancer therapeutics because it overcomes the limitation of chemotherapy, which is unable to discriminate between cancer cells and normal cells. Two genes are synthetically lethal when simultaneous disruptions of both genes gives rise to a lethal phenotype, while the disruption of either gene alone is viable. Many homologous recombination genes have synthetic lethal relationships with oncogenes and tumor suppressor genes, which can be targeted for the development of cancer therapy- an approach referred to as combination therapy. In my presentation, I will summarize recent progress in understanding both the functioning and the regulation of the DNA repair machinery and elaborate on the clinical applications of these proteins in cancer therapy.

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Transient resistance to DNA damaging agents is associated with expression of microRNAs-135b and -196b in human leukemia cell lines

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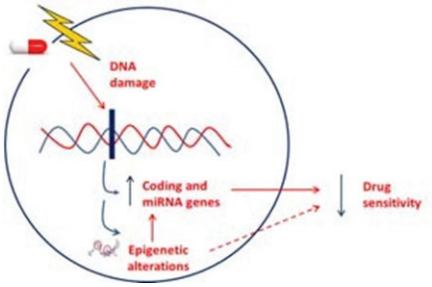

The acquisition of resistance to anticancer drugs is widely viewed as a key obstacle to successful cancer therapy. However, detailed knowledge of the initial molecular events in the response of cancer cells to these chemotherapeutic and stress responses, and how these lead to the development of chemoresistance, remains incompletely understood. Using microRNA array and washout and rechallenge experiments, we found that short term treatment of leukemia cells with etoposide led a few days later to transient resistance that was associated with a corresponding transient increase in expression of ABCB1 mRNA, as well as miR-135b and miR-196b. This phenomenon was associated with short-term exposure to genotoxic agents, such as etoposide, topotecan, doxorubicin and ionizing radiation, but not agents that do not directly damage DNA. Further, this appeared to be histiotype-specific, and was seen in leukemic cells, but not in cell lines derived from solid tumors. Treatment of leukemic cells with either 5-aza-deoxycytidine or tricostatin A produced similar increased expression of ABCB1, miR- 135b, and miR-196b, suggesting a role for epigenetic regulation of this phenomenon. Bioinformatics analyses revealed that CACNA1E, ARHGEF2, PTK2, SIAH1, ARHGAP6, and NME4 may be involved in the initial events in the development of drug resistance following the upregulation of ABCB1, miR-135b and miR-196b. In summary, we report herein that short-term exposure of cells to DNA damaging agents leads to transient drug resistance, which is associated with elevations in ABCB1, miR-135b and miR-196b, and suggests novel components that may be involved in the development of anticancer drug resistance.

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Oncofertility: Effect of chemotherapeutics and gamma tocopherol (gT) on breast cancer and primary-derived ovarian cells

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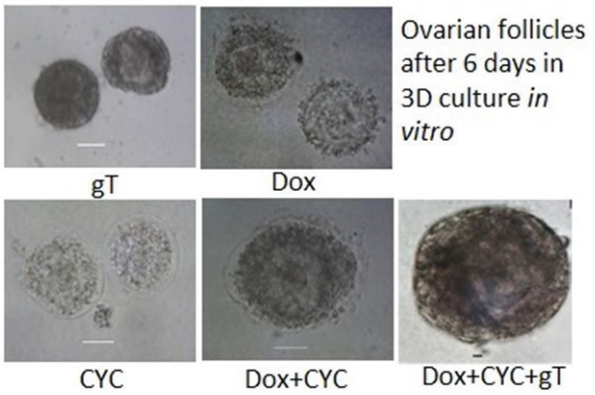
Statement of the Problem: Premenopausal breast-cancer patients are treated with a combination of chemotherapeutics, commonly doxorubicin (adriamycin) and cyclophosphamide ('AC'), but the combined toxicity of 'AC' *in vitro* has not been reported. Additionally, 'AC'-treated breast cancer survivors suffer premature ovarian failure and adverse effects caused by estrogen (E2) depletion. Each chemotherapeutic generates reactive oxygen species (ROS). Gamma- tocopherol (gT), a constituent of antioxidant Vitamin E, has anticancer activity. The aims were to examine the hypotheses that exposing a human breast cancer cell-line (MCF7) to AC+gT would reduce ROS, but gT would maintain anti-cancer activity. Secondly, that AC+gT would be less cytotoxic to primary-derived ovarian cells than 'AC'.



Methodology & Findings: MCF7 cells were exposed to doxorubicin (Dox), 4-hydroxyperoxy-cyclophosphamide (CYC), Dox+CYC, gT, and Dox+CYC+gT *in vitro*. Doxorubicin, and gT, but not CYC, caused dose- dependent cytotoxicity. Dox+CYC caused significant cytotoxicity similar to doxorubicin alone. Dox+CYC+gT caused significantly more MCF7-cell death than Dox+CYC.

Follicles (an egg surrounded by proliferating cells) from mouse ovaries were cultured in Matrigel. Follicle diameters and E2 synthesis increased under control conditions. The percentages of viable cells per follicle after 6d in 0.3% DMSO solvent control (for gT) were 60±9%, and 57±14% after exposure to the MCF7-derived EC25 value for gT. Exposure to the MCF7-derived EC25 values for Dox+CYC resulted in 16±5% ($p<0.05$, 37% of control), whereas Dox+CYC+gT (MCF7:EC25+EC25+EC25) resulted in 44±7% viable cells per follicle (74% of control).

Conclusion & Significance: Hypotheses were supported: gT increased Dox+CYC cytotoxicity against MCF7 cancer cells but decreased Dox+CYC cytotoxicity towards primary-derived proliferating ovarian cells. gT anti-cancer mechanism of action requires elucidation, but antioxidant activity may protect follicles against chemotherapeutic cytotoxicity.

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Does the PARP inhibitor Olaparib increase anti-tumor activity of the BET inhibitor JQ1 in invasive lobular carcinoma cells?

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Invasive lobular carcinoma (ILC) is the second most common sub-type of breast cancer, compromising 10% of all cases. BET inhibitors represent a novel class of anticancer agents that are clinically relevant in the treatment of ILC, however, their efficacy as single agents are limited. Recent studies demonstrate increased anti-tumour activity of BET inhibitors with the addition of a PARP inhibitor. The aim of this project was to investigate the effects of combining the BET inhibitor, JQ1, with the PARP inhibitor Olaparib, in an *in vitro* model of ILC. Molecular characterization via western blotting identified the CAMA-1 and OCUB-M cell lines as appropriate models of ILC. Initial IC50 characterization experiments completed using the MTT assay demonstrated CAMA-1 cells were more resistant to JQ1 inhibition. Subsequent analysis of the expression of BCL2, an anti-apoptotic gene, was carried out in both cell line models, with the CAMA1 cells demonstrating higher expression of this gene, suggesting an intrinsic resistance to cell death. Synergy assays performed revealed synergistic effects of combination treatment JQ1 and Olaparib. This was further shown through enhanced PARP cleavage seen in the CAMA1 cells, suggesting sensitization of CAMA1 cells to JQ1 induced death with the addition of Olaparib. In OCUB-M cells, PARP cleavage was not affected by the addition of Olaparib to JQ1. The results observed in the CAMA1 cells demonstrate the potential of sensitizing BRCA proficient cells to treatment with PARP inhibition, and the use of a combination therapy for anti-cancer treatment.

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Combination of bortezomib with olaparib decreases ovarian cancer chemoresistance

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Statement of the Problem: Ovarian cancer is one of the deadliest malignancies in women and chemoresistance is a challenge for management of ovarian cancer. In this study, we aimed to investigate the cytotoxic efficacy of combination treatment with bortezomib (inhibition of 26S proteasome) and olaparib (inhibition of poly (ADP- ribose) polymerases (PARP); PARPi) on chemosensitive and chemoresistant ovarian cancer cell lines. Methodology & Theoretical Orientation: Experiments were performed in both chemosensitive ovarian cancer cell lines (OV2008, A2780) and their chemoresistant daughter cell lines (C13, A2780-AD). Cell viability was evaluated by Sulphorhodamine B (SRB) assay following bortezomib and/or olaparib treatments with or without cisplatin.

Findings: Bortezomib and olaparib combination treatment resulted in increased cytotoxicity relative to either drug alone at certain concentrations in both chemosensitive and chemoresistant ovarian cancer cell lines. In addition, combination treatment sensitized these tumor cells to cisplatin and decreased chemoresistance at certain concentrations used.

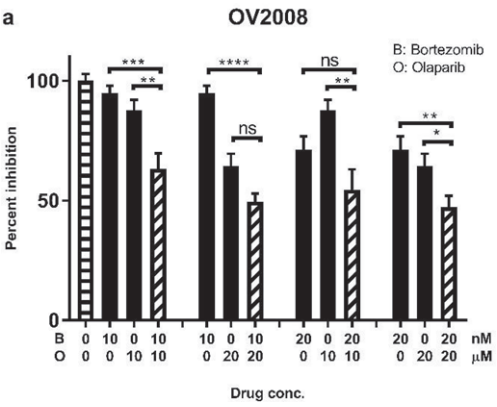

Figure 1. Representative figure of cytotoxicity of bortezomib (B) plus olaparib treatment for one of the ovarian cancer cell lines used in this study (OV2008). In this particular cell line, at B10 + O10 and B20 + O20, cell viability decreases significantly when compared to effects of either drug alone. B: bortezomib, O: olaparib.

Conclusion & Significance: This study suggests that combination of proteasome inhibition with PARP inhibition shows increased efficacy when compared to use of either drug alone in ovarian cancer cells. Based on this preclinical study, it can be assumed that administration of these agents in combination (bortezomib plus olaparib) to ovarian cancer patients may exert enhanced therapeutic effects in the clinic, improving the effect of either drug alone.

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Cytochalasin H inhibits angiogenesis via the suppression of HIF-1α protein accumulation and VEGF expression through PI3K/AKT/P70S6K and ERK1/2 signaling pathways in non-small cell lung cancer cells

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Our previous studies have isolated cytochalasin H (CyH) from mangrove-derived endophytic fungus in Zhanjiang and have demonstrated that CyH induces apoptosis and inhibits migration in A549 non-small cell lung cancer (NSCLC) cells. In this study, we further explored the effect of CyH on angiogenesis in NSCLC cells and the underlying molecular mechanisms. A549 and H460 NSCLC cells were treated with different concentrations of CyH for 24 h. The effects of CyH on NSCLC angiogenesis *in vitro* and *in vivo* were investigated. The expression of hypoxia inducible factor-1α (HIF-1α) and vascular endothelial growth factor (VEGF) in xenografted NSCLC of nude mice was analyzed by immunohistochemistry. ELISA was used to analyze the concentration of VEGF in the conditioned media derived from treated and untreated NSCLC cells. Western blot was performed to detect the levels of HIF-1α, p-AKT, p-P70S6K, and p-ERK1/2 proteins, and RT-qPCR was used to determine the levels of HIF-1α and VEGF mRNA in A549 and H460 cells. Our results showed that CyH significantly inhibited angiogenesis *in vitro* and *in vivo* and suppressed the hemoglobin content and HIF-1α and VEGF protein expression in xenografted NSCLC tissues of nude mice. CyH inhibited the secretion of VEGF protein and the expression of HIF-1α protein in A549 and H460 cells. Moreover, CyH had a significant inhibitory effect on VEGF mRNA expression but had no effect on HIF-1α mRNA expression, and CyH inhibited HIF-1α protein expression by promoting the degradation of HIF-1α protein in A549 and H460 cells. Additionally, CyH dramatically inhibited AKT, P70S6K, and ERK1/2 activation in A549 and H460 cells. Taken together, our results suggest that CyH can inhibit NSCLC angiogenesis by the suppression of HIF-1α protein accumulation and VEGF expression through PI3K/AKT/P70S6K and ERK1/2 signaling pathways.

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Insulin-like growth factor system alters the differentiation of placental mesenchymal stem cells into skeletal muscle

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Insulin-like growth factors are major components of the stem cell niche, as they regulate proliferation and differentiation of many tissues including skeletal muscle. Insulin-like growth factor binding protein-6 (IGFBP-6) is expressed in developing muscle cells and is the main regulator of IGF-II (fetal IGF). To date, no previous studies have been reported relating placental mesenchymal stem cells (PMSCs), muscle differentiation, and IGFBP-6

We hypothesized that IGFBP-6 regulates the maintenance of pluripotency of PMSCs and promotes their differentiation into muscle.

Objectives:

1) to investigate the capacity of PMSCs to differentiate into muscle; and 2) to evaluate the intracellular and extracellular actions of IGFBP-6 on PMSCs muscle differentiation.

Methods: Chorionic villi were collected from preterm placenta and stem cell identity was verified. Cells were then differentiated into muscle for 14 days and the impact of IGFBP-6 was investigated by adding IGFBP-6 or silencing it using siRNA.

Results: Isolated cells differentiated into muscle cells, forming multi-nucleated fibers expressing muscle markers with increased levels of IGFBP-6 and decreasing levels of pluripotency markers. Additionally, extracellular addition of IGFBP-6 significantly increased protein levels of muscle commitment maker Pax3/7, with an increase at the earlier time points for muscle markers that goes down by time. Interestingly, both SOX2 and OCT4 levels correlate with IGFBP-6. On the other hand, silencing IGFBP-6, significantly decreased both pluripotency and differentiation markers at the earlier time points. we identified the signal transduction mechanisms of IGFBP-6 on muscle differentiation by placental mesenchymal stem cells (PMSCs). We also showed that muscle differentiation required activation of both AKT and MAPK pathways. Interestingly, we demonstrated that IGFBP-6 could compensate for IGF-2 loss and help enhance the muscle differentiation process by triggering predominantly the MAPK pathway independent of activating either IGF-1R or the insulin receptor (IR). These findings indicate the complex interactions between IGFBP-6 and IGFs in PMSC differentiation into the skeletal muscle and that the IGF signaling axis, specifically involving IGFBP-6, is important in muscle differentiation.

Conclusion: IGFBP-6 regulates PMSC differentiation into muscle, with more prominent effects at the beginning of the differentiation process when PMSCs commit to muscle formation.

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The provenance of the JAK2 V617F exon 14 mutation in sudanese patients with chronic myeloproliferative neoplasms

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The understanding of the pathogenesis of myeloproliferative neoplasms has been aided significantly by the discovery of the JAK2 V617F exon 14 mutation and provided with additional capabilities for analysing and managing this type of disease. The aim of this study was to determine the frequency of JAK2 V617F exon 14 mutation in Sudanese patients with myeloproliferative disorders referred to Fedail Hospital and Radioisotopes Centre Khartoum (RICK) in Khartoum State-Sudan., and to investigate the differences of laboratory parameters between patients with JAK2 V617F exon 14 positive myeloproliferative neoplasms (MPNs) and JAK2 V617F exon 14 wild type MPNs.

Materials and Methods: A total of 166 patients with MPNs; 76 with polycythemia Vera (PV), 76 with essential thrombocythemia (ET) and 14 with primary myelofibrosis (PMF), and 11 healthy individuals were conducted from 2014 to 2018. DNA was isolated from peripheral blood leukocytes by QIAamp mini kit, and JAK2 V617F exon 14 mutation gene detected by quantitative real-time PCR (qRT-PCR) technology (Quant Studio 12K Flex) using TaqMan® Mutation Detection Assay and Sanger sequencing to confirm the results of TaqMan and to identify the type allele of mutations.

Results: The JAK2 V617F exon 14 was detected in 61.3% in all MPNs patients. The prevalence of JAK2 V617F exon 14 mutations was 68.6% in PV, 50% in ET and PMF patients. Mutation was not detected in 11 healthy adult people. The presence of JAK2 V617F exon 14 mutations was not associated with total WBCs count and PLTs count for PV patients, whoever the mutation positively correlates with high total RBCs count ($p = .005$), Hb concentration ($p = .018$) and HCT ($p = .016$) in PV patients, and with high total WBC count ($p = .000$) in ET patients. A JAK2 V617F exon 14 Sanger sequencing was done for 114 of the 166 patients to confirm the results of TaqMan and to identify the type allele of mutations; 64 PV, 38 ET and 12 PMF. The majority of JAK2 V617F exon 14 positive ET and PMF patients were heterozygous, while there is no JAK2 V617F exon14 homozygous allele was detected in PV patients.

Conclusions: The JAK2 V617F exon 14 mutation could be frequently detected in the Sudanese patients with MPNs, the vast majority of polycythemia patients and around half of the essential thrombocythemia and primary myelofibrosis have the mutation.

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Melatonin improves therapeutic potential of mesenchymal stem cells- derived exosomes against renal ischemia-reperfusion injury in rats

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Background: Renal ischemia-reperfusion injury (RIRI) is one of the main causes for acute kidney injury (AKI). Many previous attempts failed to adopt a suitable treatment regimen for AKI. Recently, combined melatonin (Mel) and mesenchymal stem cell (MSC)-derived exosomes (Exo) therapy gave a promising therapeutic option for acute liver ischemic injury, however this treatment approach has not been tested against RIRI yet.

Aim: This study tested the hypothesis that administration of exosomes derived from MSCs preconditioned with Mel gave best protection against RIRI as compared to therapy by MSCs or exosomes derived from non-preconditioned MSCs. Materials and Methods: Female adult rats (n = 50) equally divided into control group, sham group, RIRI group (induced by bilateral renal arteries clamping), RIRI + MSCs group (1 x10 6 bone marrow derived MSCs), RIRI + Exo group (250 µg Exo derived from no-preconditioned MSCs), and RIRI + Mel + Exo group (250 µg Exo derived from Mel preconditioned MSCs). MSCs or Exo was bilaterally injected once in each renal artery during reperfusion.

Results: The obtained results revealed notable improvement in RIRI following all treatment (MSCs, Exo, and Exo+Mel) with best improvement in Exo+Mel group as evidenced by: 1) decreased kidney injury histopathological score; 2) reduced blood levels of kidney damage markers [blood urea nitrogen (BUN) and creatinine]; 3) declined oxidative stress status (MDA level, HIF1α gene, and NOX2 protein); 4) increased anti-oxidant status (HO1 gene, and SOD, CAT, GPx activities); 5) declined apoptosis (caspase 3 activity and mRNA, and PARP1, Bax genes), 6) induced anti- apoptotic effect (Bcl2 gene); 7) inhibition of inflammation (decreased ICAM1, IL1β, NFκB and increased IL10 genes); 8) improved regeneration (bFGF, HGF and SOX9 proteins); and 9) enhanced angiogenesis (VEGF gene).

Conclusion: Treatment with exosomes derived from MSCs preconditioned with melatonin gave best protective effect against renal ischemia-reperfusion injury as compared to therapy by non-preconditioned MSCs or their exosomes.

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Receptor binding of a novel bifunctional TGF-β1/PD-L1 fusion protein elicited a down-regulated immune signature

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Statement of the Problem: There are more than 80 clinical distinct types of autoimmune diseases (AID) and their collective global prevalence rate have increased to >10%. Until now, treatment regimen has relied heavily on the use of drugs (i.e. NSAIDs, glucocorticoids and DMARDs) that down-regulate the entire body's immune response. High-dose and long-term medication with these drugs have been found to correlate with susceptibility to infections and tumorigenesis. Preclinical studies targeting the receptors of transforming growth factor superfamily such as TGF-β1 has implicated possible use of this molecule in AID management and treatment. However, researchers have reported several drawbacks of targeting TGF-β1 signaling, as they found its involvement in prevention but not reversal of AID. Another group of immune checkpoint protein, the PD-1/PD-L1 axis, has been found to down-regulate immune response and have clinical implications for treatment of the disease. This study aims to utilize the combinatorial immune-downregulating activities of TGF-β1 and PD-L1 by generating a fusion product which has never been described before.

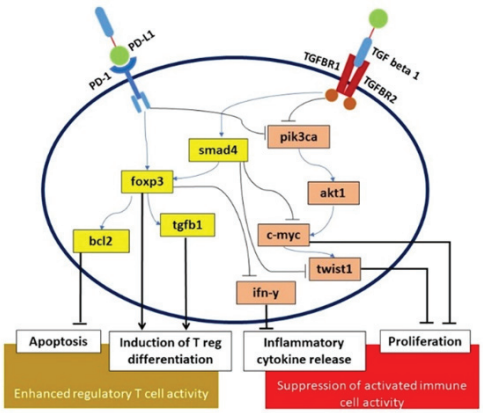

Figure 1. Down-regulated immune signature elicited by TGF-β1/PD-L1 fusion protein in AMLK cell line model as revelaed by pathway analysis using qRT-PCR.

Methodology: We were able to previously successfully clone and generate a fusion gene construct of TGF-β1 and PD-L, validated by DNA sequencing. The study focused next on characterizing the bifunctional binding of the proteins with their respective receptors by co-immunoprecipitation (co-IP) and reverse co-IP experiments coupled with pathway analysis by qRT-PCR.

Findings and Results: A 70 kDa TGF-β1/PD-L1 fusion protein was demonstrated to bind TGF-β1 receptors such as TGF-β receptor 1 and PD-L1 target receptor, PD-1, in co-IP and reverse co-IP experiments. Gene expression analysis showed that these interactions are functional and elicit gene expression signature that is seen in suppressed immunity using a cell line model.

Conclusion and Significance: TGF-β1/PD-L1 fusion protein may represent a new class of immunotherapy for treatment and management of autoimmune diseases in the future.

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