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Posters Abstracts

Zuoren Yu et al., Transcriptomics 2017, 5:2 (Suppl)
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Small RNA zippers lock miRNA molecules and block miRNA function in mammalian cells

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Small non-coding RNA-based diagnostic and therapeutic applications for human cancer are expected soon. Knocking down the expression level or blocking the function of oncogenic miRNAs is believed to be a promising strategy for cancer treatment. miRNAs loss-of-function phenotypes are mainly induced by chemically modified antisense oligonucleotides. Here, we develop an alternative inhibitor for miRNAs, termed as small RNA zipper. It is designed to bind to the 5'-half sequences of one molecule and the 3'-half sequences of another molecule of the target miRNA through a complementary interaction. The small RNA zipper can connect the target miRNA molecules end to end forming a DNA–RNA duplex with high affinity, high specificity and high stability. To avoid self-complementarity and to enhance binding specificity, LNA nucleosides were applied to synthesize the small RNA zipper. Two miRNAs, miR-221 and miR-17, were tested in human breast cancer cell lines, demonstrating the 70%-90% knockdown of miRNA levels by 30–50 nM small RNA zippers at 24-48 h after transfection. The effect of the miRNA zipper was not limited to the target miRNA, but also to the expression of iso-miRNAs. Let-7 family members and point mutated sequence were applied to further validate the specificity of the miRNA zippers. The miR-221 zipper shows capability in rescuing the expression of target genes of miR-221 and reversing the oncogenic function of miR-221 in breast cancer cells. In addition, we demonstrate that the miR-221 zipper attenuates doxorubicin resistance with higher efficiency than anti-miR-221 in human breast cancer cells. Taken together, small RNA zippers are a novel type of miRNA inhibitors, which can be used to induce miRNA loss-of-function phenotypes and validate miRNA target genes.

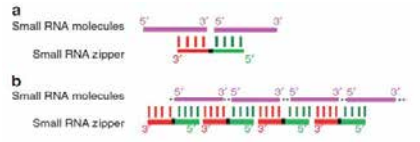

Figure 1 | The construction of small RNA zipper. (**a**) Schematic representation of the interaction between small RNA zipper and small RNAs. (**b**) Schematic representation of how the small RNA zippers connect the target small RNA molecules end to end, forming an RNA-DNA duplex.

Biography

Zuoren Yu is currently a Professor at Tongji University School of Medicine, Research Center for Translational, Shanghai East Hospital. After obtaining PhD degree in 2003 from Chinese Academy of Medical Sciences, he started Post-doctoral training at University of Pennsylvania School of Medicine and Thomas Jefferson University Kimmel Cancer Center. In 2009, he was assigned for a Faculty position in the track of Research Assistant Professor at Thomas Jefferson University. After joining Tongji University in Shanghai in 2012, he has been focusing on non-coding RNA regulation of breast cancer stem cells related with drug sensitivity, tumour regeneration and cancer metastasis. His main research work includes: Finding cell cycle regulator CCND1 is involved in the regulation of miRNA biogenesis and histone H3K9 tri-methylation, finding a regulatory loop between CCND1 and miR-17/20 in breast cancer, and demonstrating a microenvironment-mediated heterotypic signalling through which miR-17/20 regulate cross talking between cancer cells and inhibit breast cancer cell migration and metastasis.

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Sarah Eysoldt et al., Transcriptomics 2017, 5:2 (Suppl)
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Phosphorylated oximes increase organophosphate toxicity

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Aim: Oximes are small chemical compounds utilized as treatments for organophosphate toxicity. Organophosphorus nerve agents prevent the enzyme acetylcholinesterase from performing its function, which is breaking down the neurotransmitter, acetylcholine. Nerve agents inhibit acetylcholinesterase by bonding their phosphate group to acetylcholinesterase. Oximes are used to remove the phosphate group from the nerve agent, allowing it to detach, therefore restoring the function of acetylcholinesterase. However, when this takes place, we end up with by-product known as phosphorylated oxime. Phosphorylated oximes may be dangerous because they can inhibit acetylcholinesterase more potently than organophosphates; resulting in toxicity rather than a cure. The objective of this study is to evaluate inhibitory capacity and the toxicity of phosphorylated oximes to mammalian cells.

Methods: The series of experiments conducted involved varying amounts of different oximes (K027, 2-Pralidoxime, etc.) and organophosphates (asinphos, dicrotophos, etc.) on NIH-3T3 and SH-SY5Y cells. Experiments included in-cell westerns to measure amounts of acetylcholinesterase levels, a colorimetric assay to measure acetylcholinesterase activity, and other measures of toxicity. An on-cell western blot was also developed to assess the number of acetylcholinesterase receptor neuronal cells. These experiments will examine the contributions of oximes, organophosphates, and the combination of both chemicals on acetylcholinesterase function and off- target toxicity.

Results: The results of this study suggest that the combination of nerve agents and oximes increases toxicity within neuronal cells. A colorimetric assay showed a significant decrease in the activity of acetylcholinesterase when the combination of dicrotophos and 2- PAM was added compared to dicrotophos alone. Measures of mitochondrial toxicity using the XF- 96 Flux Analyzer also showed that the combination of dicrotophos and 2- PAM detrimentally affected the cells even more than the nerve agent alone. More experiments are currently being developed to further investigate this phenomenon, and potentially explain the molecular process of this potent inhibition of acetylcholinesterase.

Conclusion: From these results, it can be determined that oximes are not a safe and viable treatment option for organophosphate toxicity because the combination of the two does more harm than good in NIH- 3T3 cells.

Discussion: The research findings in this study have the potential to change the course of how organophosphate intoxication is treated. Our goal is to improve the treatment of organophosphate toxicity to prevent the recent tragedies in Syria and Iraq from occurring in the future.

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Anne Li, Transcriptomics 2017, 5:2 (Suppl)
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A predictive model for methionine oxidation propensity in therapeutic protein

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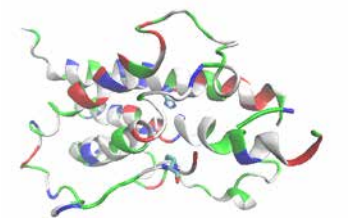
Methionine is one of the amino acids that are subjected to oxidation, and can subsequently lead to loss of molecular bioactivities or induce certain health conditions. Typically multiple methionine residues are present in protein molecules, and their oxidation characteristics can be dramatically different. The secondary structures, hydrophobicity, local chain flexibility, and solvent accessibility are all contributing factors to the oxidation propensity of these methionine residues. In this work, a predictive model has been developed to correlate the methionine residual oxidation propensities with their aforementioned properties. The model proteins used in this study, including monoclonal antibodies, growth hormone, interferons, etc., have all been investigated experimentally on their methionine oxidation propensity, and well resolved protein crystal structures documented in RCSB Protein Data Bank (PDB). The crystal structures of these proteins were optimized using QwikMD simulation program (UIUC) to produce well solvated structures. These properties of each methionine residue in the proteins, including hydrophobicity, local chain flexibility, and solvent accessibility, etc., were quantified and a mathematical model was developed to correlate them with the rank ordering of their oxidation propensities reported in literatures. The model, once further validated, provides a qualitative prediction tool to rank order methionine oxidation propensity in protein molecules that are complementary to experimental investigation approaches.



Biography

Anne Li is a Senior at Wootton High School with interest in Biological Chemistry. She takes an active role in the STEM field at her schools as the President of both Chemistry Club and Biology Club. Through her experience with the National Chemistry Olympiad and internship at the National Foundation for Cancer Research, she has been able to apply what she has learned in the classroom to help the mission to find a cure for cancer.

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Reham Abo Elwafa et al., Transcriptomics 2017, 5:2 (Suppl)
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Study of alternative *Wilms tumor* gene methylation as an epigenetic biomarker in acute myeloid leukemia

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Background: Overexpression of the *Wilms tumor 1 gene* (*WT1*) is implicated in the prognosis of acute myeloid leukemia (AML) with high expression predicting disease progression, as well as being intensively studied as a potential molecular marker for minimal residual disease (MRD) and treatment response. Many different isoforms for *WT1* are generated by alternative transcription initiation, mRNA splicing and alternative translation initiation. Recently, an alternative promoter incorporating a unique first exon, alternative *WT1* transcript *(AWT1*), has been described. The *AWT1* expression and the underlying epigenetic alterations associated with its expression in AML are still unknown.

Objectives: We studied the *AWT1* gene specific methylation changes and its relationship with other clinicopathological features. We also integrated the corresponding gene expression profile to explore the role of methylation in regulating gene expression.

Materials & Methods: Bisulfite PCR followed by pyrosequencing were done to determine the methylation status of *AWT1* gene promotor CPG islands in 50 newly diagnosed AML patients and 50 healthy subjects as a control group. The level of *AWT1* expression was assessed using RQ-PCR.

Results: *AWT1* expression level was significantly higher in the AML patients in comparison to the control group (P<0.001) and it was surprising to find robust hypermethylation of the *AWT1* promoter in AML patients compared to the controls (P<0.001). A statistically significant negative correlation between *AWT1* expression and methylation level was found (r=0.67, P<0.001). At a cutoff value of 45.2% *AWT1* promoter hypermethylation was found to be a highly specific marker for AML (specificity 95% and sensitivity 97.5%)

Conclusion: We described an expression methylation signature of the *AWT1* that are promising markers for diagnosis and MRD assessment in AML.

Biography

Reham Abo Elwafa is a Lecturer of Clinical Pathology, Faculty of Medicine in Alexandria University, Egypt. She has expertise in Research, Teaching and Administration in Hospital and Education Institution. She is expert in Molecular Techniques: PCR (conventional, and real time PCR), microarray, pyrosequencing and NGS in addition to FISH techniques including Prenatal Genetic Diagnosis (PGD) and flow cytometric immunophenotyping of different types of hematologic malignancies. She has several international publications in the field of Molecular Biology, Genetics and Epigenetics.

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Yojiro Ishida et al., Transcriptomics 2017, 5:2 (Suppl)
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A CUGGU/UUGGU-specific MazF homologue from *Methanohalobium evestigatum*

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MazF is a sequence-specific endoribonuclease or mRNA interferases, which cleaves RNA at a specific sequence. Since the expression of a specific gene or a group of specific genes can be regulated by MazF, expanding the repertoire of recognition sequences by MazF mRNA interferases is highly desirable for biotechnological and medical applications. Here, we identified a gene for a MazF homologue (MazFme) from *Methanohalobium evestigatum*, an extremely halophilic archaeon. In order to suppress the toxicity of MazFme to the E. coli cells, the C-terminal half of the cognate antitoxin MazEme was fused to the N-terminal ends of MazFme. After purification of the MazEme-MazFme fusion protein, MazFme was released from the fusion protein by factor Xa treatment. The free MazFme RNA cleavage specificity was determined by primer extension and synthetic ribonucleotides, revealing that MazFme is a CUGGU/UUGGU-specific endoribonuclease.

Biography

Yojiro Ishida has recently obtained his PhD from Hiroshima University. His mentors are Professor Tadashi Shimamoto, Hiroshima University and Professor Masayori Inouye, Rutgers University. He has developed a new expression system to incorporate a toxic amino acid analogue into a protein to alter the function by using the single-protein production (SPP) system. Furthermore, he developed a residue and stereo specific labeling system for NMR structural studies. Currently, his research focus are: Discovery of new MazF homologues, application of MazF for specific gene regulations, characterization of new Toxin-Antitoxin (TA) systems from *Staphylococcus aureus*, and incorporating ^{19}F probe into methyl groups of a protein to characterize large molecular weight proteins and membrane proteins using the SPP system in *E. coli*.

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RNA-templated DNA double-strand break repair: Role of RAD52

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Statement of the Problem: Homologous recombination (HR) is a high-fidelity process that uses homologous DNA sequences as a template to repair damaged DNA. However, we recently demonstrated that transcript RNA can also serve as template for DSB repair via HR in yeast. Currently, little is known about the enzymatic machinery that executes RNA-templated DSB repair. Our results from budding yeast implicated Rad52 in this RNA-directed DSB repair mechanism. However, the exact mechanism of how RAD52 contributes to RNA-dependent DSB repair remains to be elucidated.

Methodology & Theoretical Orientation: Using biochemical and genetic approaches in yeast we investigate this mechanism.

Findings: We found that RAD52 carries inverse strand exchange activity between homologous dsDNA and ssRNA, which could account for the role of RAD52 in RNA-dependent DNA repair identified in our genetic experiments. This activity is distinct from canonical "forward" DNA strand exchange which is carried by the major recombinase RAD51 between ssDNA and homologous dsDNA. We demonstrate that both human and yeast RAD52 efficiently promotes inverse strand exchange between dsDNA and homologous ssRNA or ssDNA. We show that in eukaryotes, inverse RNA strand exchange is a distinctive activity of RAD52; neither the major recombinase RAD51, nor the yeast RAD59 carries this activity. Our genetic experiments in yeast support the biological significance of inverse RNA strand exchange.

Conclusion & Significance: It was demonstrated that RAD52 inactivation causes synthetic lethality in combination with mutations in BRCA1 and BRCA2 proteins, defects of which are associated with various types of cancer. These data indicated an essential back-up function of RAD52, which may complement the BRCA-dependency in humans. We suggest that the novel RAD52 inverse strand exchange activities contribute to this back-up function. Thus, our findings may help to identify new therapeutic targets for cancer.

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Using single-stranded DNA for homology directed repair catalyzed by CRISPR/Cas9 activity

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This workshop session will feature many speakers who are actively working in the field of gene editing with a specific focus on the use of single-stranded DNA templates in combination with gene editing tools such as CRISPR/Cas9 to repair genetic mutations via homology directed repair. Speakers will describe the design and rationale for using varying types of single-stranded DNA molecules, ranging from short single-stranded oligonucleotides to long strands generated by multiple amplifications in vitro. The overarching objective of all these projects is to reverse a point mutation or to restore gene function by homologous fragment insertion. Each speaker will also detail experimental case studies in which their method of choice was either successful or unsuccessful in generating the predicted genetic outcome. What were the consequences of these failed attempts? The use of single-stranded DNA functioning as patching or bridging the resected section of DNA created by the double-stranded break directed by CRISPR/Cas9 to reduce allelic heterogeneity will also be discussed. The format of the workshop will be interactive and a healthy dose of participation by attendees will be highly encouraged. We hope to achieve an understanding of how to study homology directed repair in mammalian cells in the most effective way, both transformed and primary. We hope to define what is real, reproducible and robust? And what is also non-reproducible artefactual or fictional? so that gene editing can grow in a truly healthy fashion, driven by science and not by publicity.

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Aptamers selection methodology and strategy based on multiple modes of capillary electrophoresis

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Nucleic acid aptamers are short, single-stranded DNA (ssDNA) or RNA molecules that are selected for binding to a specific target. Aptamers can be used as recognition probes in biomedical, food and environment analysis. Moreover, they have great potential in disease diagnosis and treatment, drug discovery, medicine research, as well as bioimaging, which are expected to bring huge economic benefits. However, current aptamers application is far from satisfactory, and has not yet been fully developed. The complicated selection process with high cost and low efficiency is one of the bottlenecks of their application. Still universally accepted standard selection methods are not accepted. Capillary electrophoresis (CE) is one of the most powerful methods for aptamers sieving (known as CE-SELEX), which has the advantages of fast, high resolution, low sample consumption and smart separation modes of capillary zone electrophoresis (CZE), affinity capillary electrophoresis (ACE) and capillary isoelectric focus (CIEF). Moreover, the binding of target and synthetic single stranded DNA (ss-DNA) occurs in free solution, which eliminates the biases caused by stationary support and linker. Some important protein aptamers have been successfully obtained based on CE, which greatly improves the selection efficiency. In our group, we aim at selection strategy research of aptamers against multiple targets based on capillary electrophoresis, which has the characteristics of high efficiency, high speed, low cost and multiple available modes. We propose selection strategies including: the availability of randomly synthesized ssDNA libraries should be evaluated, the binding evaluation can be made based on fast CE analysis, which provides more information to guide further selection.

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Transposon-mediated directed mutation in *E. coli*

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Escherichia coli cells deleted for the cyclic AMP (cAMP) receptor protein (Crp) gene (Δcrp) cannot utilize glycerol because cAMP-Crp is a required activator of the glycerol utilization operon, glpFK. We have previously shown that a transposon, Insertion Sequence 5 (IS5), can insert into the upstream regulatory region of the operon to activate the glpFK promoter and enable glycerol utilization. GlpR, which represses glpFK transcription, binds to the glpFK upstream region near the site of IS5 insertion and inhibits insertion. By adding cAMP to the culture medium in ΔcyaA cells, the cAMP-Crp complex, which also binds to the glpFK upstream regulatory region, inhibits IS5 hopping into the activating site. Control experiments show that the frequencies of mutations in response to cAMP were independent of parental cell growth rate and the selection procedure. These findings led to the prediction that glpFK-activating IS5 insertions can also occur in wild-type (Crp+) cells under conditions that limit cAMP production. Accordingly, IS5 insertion into the activating site in wild-type cells is elevated in the presence of glycerol and a non-metabolizable sugar analogue that lowers cytoplasmic cAMP concentrations. The resultant IS5 insertion mutants arising in this minimal medium become dominant constituents of the population after prolonged periods of growth. Thus, DNA binding transcription factors can reversibly mask a favored transposon target site, rendering a hot spot for insertion less favored. Such mechanisms could have evolved by natural selection to overcome environmental adversity. We have further shown that IS elements can insert upstream of the flagellar master regulator operon, flhDC, to activate transcription in a process that depends on viscosity (agar concentration). Documentation of these processes shows that IS elements can direct mutations (IS insertions) to specific sites in response to environmental stress.

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FOXO1/Sprouty-2 pathway inhibits endothelial cell tumor growth

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Vascular tumors are neoplasms of endothelial cell origin and have a wide spectrum of clinical presentations, ranging from benign infantile hemangiomas in children to low-grade malignant hemangioendotheliomas and highly aggressive angiosarcomas in adults. To date, the molecular basis of vascular tumor pathogenesis is poorly understood and standard therapy for these tumors has limited clinical efficacy. Forkhead box protein O1 (*FOXO1*) is a transcription factor with tumor suppressor function and is dysregulated in human cancer. In this study, we showed that *FOXO1* suppressed vascular tumor growth, and mechanistically, the inhibitory effects of *FOXO1* are mediated by Sprouty2. *FOXO1* expression was reduced in a variety of human vascular tumors examined. Knockdown of *FOXO1* gene expression with short hairpin RNA resulted in increased vascular tumor cell migration and proliferation in vitro and in vivo animal models. Conversely, over-expression of constitutively active *FOXO1* in these cells suppressed cell growth. We observed that *FOXO1* interacted with Sprouty2 promoter in situ in chromatin immunoprecipitation assay and increased Sprouty2 gene expression in tumor cells. Like FOXO1, Sprouty2 expression was reduced in vascular tumors. Over-expression of Sprouty2 decreased tumor cell growth and migration. Conversely, knockdown of Sprouty2 increased tumor growth in vitro and in vivo. Knockdown of Sprouty2 in cells with over-expression of constitutively active *FOXO1* resulted in reduced tumor growth and rescued the *FOXO1* phenotype, indicating that Sprouty2 is an important mediator of the biological effects of FOXO1. Microarray gene expression profiling of human angiosarcoma cells with Sprouty2 knockdown together with network data integration using bioinformatics analysis revealed important Sprouty2-regulated genes that are involved in angiogenesis, apoptosis and growth signal transduction pathways. In summary, these findings demonstrate important growth regulatory role of the FOXO1/Sprouty2 pathway in endothelial cell tumors and highlight the potential roles of novel pathways downstream of Sprouty2 in these lesions.

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Analyzing cancer genomics using watson for genomics with structural variants

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Statement of the Problem: As sequencing cost declines, more cancer patients have their tumor samples sequenced to seek for optimum treatments. However, analyzing genomic data requires considerable expertise and efforts. Besides, reports should be generated in clinically relevant time frame, without errors, with objectivity, and in comprehensive manner. It is difficult to meet those criteria in ever increasing flood of new publications, clinical trial information on investigational drugs, and more high-resolution data. As an example of high resolution data, structural variant data is available. However, so far, fusion proteins such as BCR-ABL, EML4-ALK receives attentions; other types of structural variants such as simple disrupts, exon skippings, intron retentions, and internal tandem duplications are underestimated.

Methodology & Theoretical Orientation: Watson for Genomics (WfG) takes variants, copy number alterations, and gene expressions as inputs to generate a report in automated manner. WfG performs molecular profile to identify driver mutations and drug response biomarkers followed by drug analysis including pathway analysis. Structural variants module is being developed to accommodate structural variant data.

Findings: WfG generates reports with high recall rates in drug recommendation compared with human experts using whole genome samples from collaborators such as New York Genome Center and British Columbia Cancer Agency. Structural variants such as EGFR vIII, MET exon skipping event, and tumor suppressor gene disruptions are successfully captured along with de novo events. WfG provides cancer communities with up to date knowledge to benefit their patients.

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Biochemical implications of administration of halofantrine hydrochloride (Halfan) on estradiol levels of female Wistar rats

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This study determines the effects of doses of halofantrine hydrochloride, a phenanthrene methanol drug used in the therapeutic treatment of malaria on the estradiol levels of female Wistar rats. A suspension of drugs at a dose of 0.2 ml/kg body weight three times at six hourly intervals were administered orally to different groups of mature female rats for 2 weeks, 4 weeks and 6 weeks duration, control groups received similar treatment doses of normal saline. The animals were sacrificed on the 14th day, 28th day and 42nd day respectively after drug administration by cervical dislocation. Whole blood samples were collected for full blood count: (WBC, RBC, PCV, Hg and platelets counts). From the plasma, hormonal level was determined by radio-immunoassay, the activities of AST, ALT, ALP, TB and CB, lipid profile test: (TC, TG, HDL-C, LDL-C) and Kidney profile test: (urea, creatinine, BCO3, Na, K and Cl) were also determined. The level of estradiol level following 2 weeks, 4 weeks and 6 weeks treatment was higher significantly ($p<0.05$) in all the groups compared to the control. The activities of AST, ALT, ALP, TB, CB, TC, TG, HDL-C, and LDL-C increased significantly ($p<0.05$). The full blood counts and kidney profile tests increased in a dose dependent manner. These findings could signify toxicity of the drugs on the bone marrow of the rats, the increase in the full blood count indicates a pathological condition and renal dysfunction for the kidney profile tests. The drug was also discovered to cause increase in the serum enzyme levels in the experimental rats, suggesting a possible hepatotoxicity of this drug.

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Panoramix: The missing link between the piRNA pathway and the general silencing machinery

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The piwi-interacting RNA (piRNA) pathway is a small RNA-based innate immune system that defends germ cell genomes against parasitic transposons. In Drosophila ovaries, the nuclear piwi protein is required for transcriptional silencing of transposons, though the precise mechanisms by which this occurs are unknown. Through mining the data from several independent genome-wide RNAi screens for factors required for transposon silencing, we identified an ovary specific nuclear protein (CG9754/Panoramix) that can influence global transposon transcription similarly as piwi when eliminated. The effect is not due to the defects of piRNA biogenesis since levels of piRNAs remained unchanged and piwi proteins stayed bound with piRNAs in nucleus. Strikingly, enforced tethering of this protein to nascent mRNA transcripts, causes co-transcriptional silencing of the source locus (~1000-fold repression) and the deposition of repressive chromatin marks. Interestingly, this protein is a component of piwi complexes that functions downstream of piwi and its binding partner, Asterix. We have named this gene Panoramix, the mentor who empowers Asterix to perform his feats of strength. Importantly, we found that both Eggless/dSetDB1 (H3K9 methyltransferase) and dLSD1 (H3K4me2 demethylase) are required for Panoramix-mediated silencing. Therefore, we propose that Panoramix forms one of the missing links between the piRNA pathway and the general silencing machinery that it recruits to enforce transcriptional repression to protect germline from transposons.

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miR-375-3p negatively regulates osteogenesis by targeting LRP5 and β-catenin

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Wnt signaling pathways are essential for bone formation. Previous studies showed that Wnt signaling pathways were regulated by miR-375. Thus, we aim to explore whether miR-375 could affect osteogenesis. In the present study, we investigated the roles of miR-375 and its downstream targets. Firstly, we revealed that miR-375-3p negatively modulated osteogenesis by suppressing positive regulators of osteogenesis and promoting negative regulators of osteogenesis. In addition, the results of TUNEL cell apoptosis assay showed that miR-375-3p induced MC3T3-E1 cell apoptosis. Secondly, miR-375-3p targeted low-density lipoprotein receptor-related protein 5 (LRP5), a co-receptor of the Wnt signaling pathways and β-catenin as determined by luciferase activity assay and it decreased the expression levels of LRP5 and β-catenin. Thirdly, the decline of protein levels of β-catenin was determined by immunocytochemistry and immunofluorescence. Finally, silence of LRP5 in osteoblast precursor cells resulted in diminished cell viability and cell proliferation as detected by WST-1-based colorimetric assay. Additionally, all the parameters including the relative bone volume from μCT measurement suggested that LRP5 knockout in mice resulted in a looser and worse-connected trabecula. The mRNA levels of important negative modulators relating to osteogenesis increased after the functions of LRP5 were blocked in mice. Finally, the expression levels of LRP5 increased during the osteogenesis of MC3T3-E1, while the levels of β-catenin decreased in bone tissues from osteoporotic patients with vertebral compression fractures. In conclusion, we revealed miR-375-3p negatively regulated osteogenesis by targeting LRP5 and β-catenin. In addition, loss of functions of LRP5 damaged bone formation *in vivo*. Clinically, miR-375-3p and its targets might be used as diagnostic biomarkers for osteoporosis and might be also as novel therapeutic agents in osteoporosis treatment. The relevant products of miR-375-3p might be developed into molecular drugs in the future. These molecules could be used in translational medicine.

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