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Posters

Epigenetics 2017

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Potential new role for ROS in the epigenetic regulation of gene expression

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The generation of Reactive Oxygen Species (ROS) as by-products of the highly efficient aerobic metabolism constitute an inescapable biochemical side effect that can be extremely harmful for cell viability, due to the irreversible oxidation of lipids, proteins and nucleic acids. Nevertheless, eukaryotic cells can also actively generate ROS as essential components of molecular mechanisms regulating key cellular processes, including proliferation and differentiation, through the oxidation of redox-sensitive proteins such as kinases and phosphatases. ROS production in ESCs is low as anaerobic glycolysis rather than oxidative phosphorylation (OxPhos) is favoured. A switch from glycolysis to OxPhos is observed during ESC differentiation and accumulating evidence suggests that ROS is an important signalling molecule for ESC differentiation. Here we propose that eukaryotic cells could use ROS to directly regulate DNA methylation and gene expression patterns through the oxidation of methylated cytosines at target gene promoters.

To test this hypothesis, we have used a Protoporphyrin IX-dependent photodynamic treatment (PT) tool to activate a transient production of non-lethal ROS levels in the feeder-independent E14 mouse embryonic stem cell line. Using this tool, we report that the endogenous production of non-lethal ROS levels promotes a cytosine demethylation of tested promoters suggesting that these oxygen derivatives may be involved in the regulation of gene expression patterns through the dynamic modulation of DNA methylation patterns.

Recent Publications

1. Thomson JP, Meehan RR. The application of genome-wide 5-hydroxymethylcytosine studies in cancer research. Epigenomics 2017, 9:77-91
2. Cole JJ et al. Diverse interventions that extend mouse lifespan suppress shared age-associated epigenetic changes at critical gene regulatory regions. Genome Biol 2017, 18:58.
3. Thomson JP et al. Loss of Tet1-Associated 5-Hydroxymethylcytosine Is Concomitant with Aberrant Promoter Hypermethylation in Liver Cancer. Cancer Res 2016, 76:3097-3108.
4. Nestor CE et al. 5-hydroxymethylcytosine remodeling precedes lineage specification during differentiation of human CD4+ T-cells. Cell Reports 2016, 16:559-570.
5. Carrasco E et al. Switching on a transient endogenous ROS production in mammalian cells and tissues. Methods. 109:180, 2016.
6. Fonda-Pascual P et al. In situ production of ROS in the skin by PDT as a powerful tool in clinical dermatology. Methods. 109:190, 2016.
7. Carrasco E et al. Photoactivation of ROS Production in Situ Transiently Activates Cell Proliferation in Mouse Skin and in the hair Follicle Stem Cell Niche Promoting Hair Growth and Wound Healing. Journal of Investigative Dermatology. 135:11, 2015.
8. Nestor CE et al. Rapid reprogramming of epigenetic and transcriptional profiles in mammalian culture systems. Genome Biol 2015, 16:11.
9. Blázquez-Castro A et al. Protoporphyrin IX-dependent photodynamic production of endogenous ROS stimulates cell proliferation. European Journal of Cell Biology. 91:216, 2012.
10. Juarranz A et al. Mitotic catastrophe is implicated in the resistance of basal carcinoma cells to photodynamic therapy. Journal of Investigative Dermatology. 2012.

11. Espada J et al. Regulation of SNAIL1 and E-cadherin function by DNMT1 in a DNA methylation-independent context. Nucleic Acids Research. 39:9194, 2011

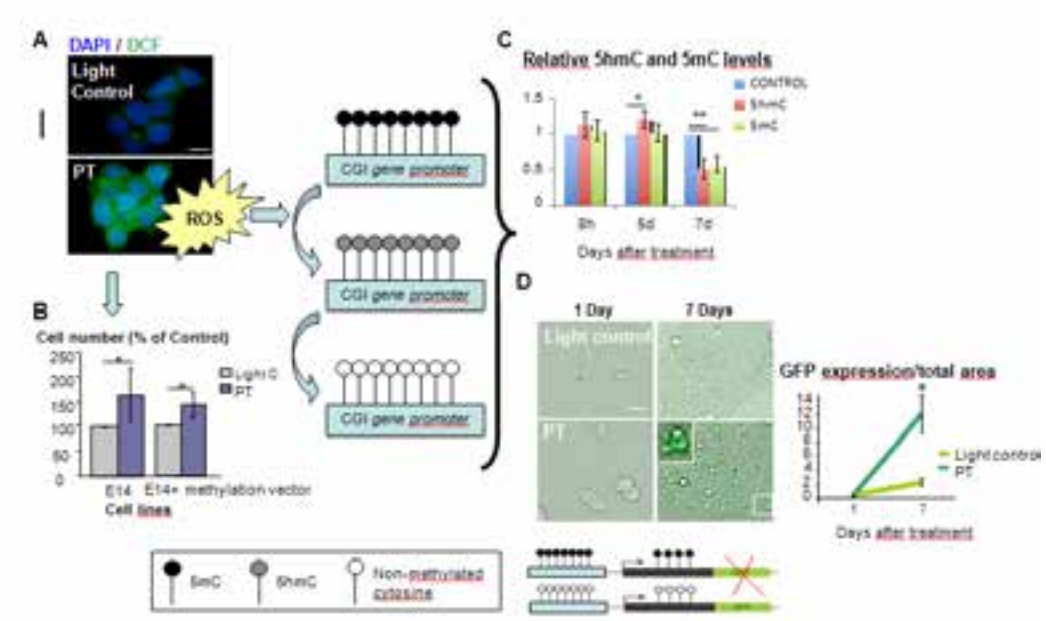

*Figure 1. Potential new role for ROS in the epigenetic regulation of gene expression (A) One single dose of PT generates non lethal ROS levels (DCF detection) that have physiological effects such as the stimulation of cell proliferation (B), but also could have other roles as the regulation of DNA methylation and gene expression, through the oxidation of the 5mC in CGIs. (C) DNA Dot Blot analysis quantification shows that 5 days after PT, there is an increase in 5hmC levels, followed by a decrease in 5hmC and 5mC levels 7 days after, compared to light control cells. (D) To test if the global reduction of 5mC levels is correlated to a change in transcription levels, a stable transfection with a methylation reporter gene (that expresses GFP when unmethylated) was performed. From 5 to 7 days after PT, a significant and gradual increase in GFP expression was detected, as shown in the images and in the graph. All images are representative of at least three independent experiments. Scale bar: 10 and 40 µm, A and D, respectively. The mean ± SD values of at least three independent experiments are represented in each graph. **: Significant, P ≤ 0.01. *: Significant, P ≤ 0,1.*

Biography

María Inmaculada Calvo Sánchez holds a postdoc position in the Experimental Dermatology and Skin Biology Group of the IRYCIS, Ramón y Cajal University Hospital. The research of this group is focused on cutaneus regenerative medicine and epigenetic changes induced by reactive oxygen species. Also she collaborates with the Francisco de Vitoria University as an associate professor in the degree of Biotechnology and in the Master's Degree in Advanced Therapies and Biotechnology Innovation, and as a coordinator of the practical part of the Masters mentioned above.

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mRNA expression of the glutathione related genes in cultured HeLa cells treated with valproic acid

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Statement of the Problem: The greatest challenge for anticancer therapy is the tendency of malignant cells to develop multidrug resistance (MDR). This phenomenon is manifested as a decrease in drug uptake, an increase in drug efflux by ABC transporters, and the activation of detoxification systems (phase II metabolism, the glutathione system), among other mechanisms of the MDR phenotype. Recently, a new group of anticancer drugs known as epigenetic agents, histone deacetylase inhibitors (HDACIs), has shown better clinical results. They are more specific and less toxic, and are capable of sensitizing malignant cells to conventional anticancer drugs. However, HDACIs have also proven to induce the MDR phenotype. Since glutathione (GSH) is the main detoxification system present in all tissues, the present study focuses on the relation between treatment with valproic acid (an epigenetic drug) and the expression of GSH-related genes in HeLa cell cultures.

Methodology & Theoretical Orientation: Firstly, the molecular analysis of mRNA expression was made by standard PCR, using the total RNA obtained from Hela cells. Then reverse transcription to cDNA was performed and a viability assay was made by using the MTT protocol to evaluate the effects of VPA and BSO (Buthionine sulfoximine), alone or in combination. The second step was to measure the level of intracellular GSH, previously exposing the cells to treatment with VPA and BSO.

Findings: A significant increase was found in the mRNA expression of the glutamate-cysteine ligase modifier subunit (GCLM) and glutathione synthase (GSS). The MTT assay showed a decrease in cell viability with the use of VPA and even more so with the combination of VPA+BSO. The measurement and analysis of variation in the level of GSH is still in progress.

Conclusion & Significance: The induction of MDR by VPA can be considered as mild. This agent may be useful in the treatment of tumors because it can induce death in cancer cells, alone or in combination with BSO or other anticancer drugs. Further studies are necessary to analyze the level of proteins participating in glutathione biosynthesis and recycling after VPA treatment.

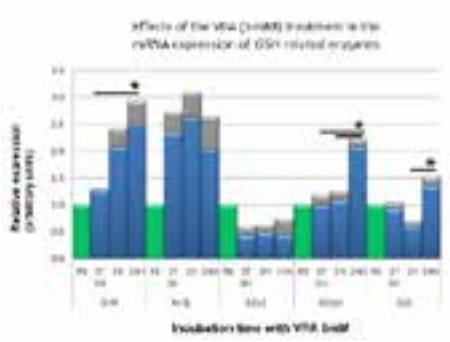

Figure 1: Effects of the VPA (5 mM) treatment in the mRNA expression of GSH related enzymes along time. Relative expression is shown in the y axis. Ribosomal RNA was used as positive control (green), comparisons were made between time zero, 2 and 24 hours of VPA exposition.

Recent Publication

1. Papanikolopoulou A, Syrigos KN and Nikolaos Drakoulis (2015) The role of glutamine supplementation in thoracic and upper aerodigestive malignancies. Nutrition and Cancer. 67(2):231-237.
2. Gul K, Muge A, Taner A, et al (2015) Oral glutamine supplementation reduces radiotherapy- induced esophagitis in lung cancer patients. Asian Pac J Cancer Prev. 16(1):53-58.

3. Tsujimoto T, Yamamoto Y, Wasa M, et al (2015) L-glutamine decreases the severity of mucositis induced by chemoradiotherapy in patients with locally advanced head and neck cancer: a double-blind, randomized, placebo-controlled trial. Oncol Rep. 33(1):33-39.
4. Chattopadhyay S, Saha A, Azam M, et al (2014) Role of oral glutamine in alleviation and prevention of radiation-induced oral mucositis South Asian Journal of Cancer 3(1):8-12
5. Tutanc OD, Aydogan A, Akkucuk S, et al (2013) The efficacy of oral glutamine in prevention of acute radiotherapy-induced esophagitis in patients with lung cancer. Contemporary Oncology (Poznan) 17(6):520-524.

Biography

Benjamin Gonzalez Lopez has his expertise and passion in development of morphologic techniques, cell culture and molecular biology. He is a medic graduated in 2014 from Instituto Politecnico Nacional, Mexico City, now studying his 2nd year of master's degree in morphology at the same institution, interested in improving health and wellbeing of people in general, responsible and committed as a student, motivated for the acquisition of new knowledge and skills.

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Raquel Arminda Carvalho Machado et al., J Clin Epigenet 2017, 3:4
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Molecular cloning and analysis of the CHD7 chromatin remodeler promoter region

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[2]Dana Farber Cancer Institute-Harvard Medical School, USA

Statement of the Problem: Glioblastoma (GBM) is the most common, aggressive and fatal type of cerebral tumor. The average patients survival rate is 12-15 months, highlighting the urgent need for more effective targeted therapeutics. CHD7 is an ATP-dependent chromatin remodeler protein that functions in enhancer mediated transcription. Therefore, abnormal CHD7 expression may result in aberrant transcription of tissue-specific genes. Previous results from our laboratory suggest that the CHD7 gene is highly expressed in glioma patient samples, when compared to normal brain tissue. However, the mechanism underlying this overexpression is still not understood. In this study, we aimed to identify the CHD7 promoter region and test different signaling pathways that may directly modulate the expression of this gene in GBM cells.

Methodology & Theoretical Orientation: To predict the region with promoter activity, we analyzed the CHD7 gene sequence using the NCBI database. Based on this analysis, we selected the -1149/+619 fragment as a candidate for the CHD7 core promoter region. We then cloned the sense and antisense sequences into the pGL3 basic vector. The recombinant plasmids and internal control of marine intestine luciferase expression vector were transiently transfected into 293T cells for validation.

Findings: By using the luciferase reporter gene assay, we identified a regulatory region of 1.7 kbp for CHD7. This fragment greatly stimulated luciferase activity by 15-fold, when compared to the empty vector. The antisense sequence did not show significant activity, indicating that CHD7 expression is regulated by a unidirectional promoter.

Conclusion & Significance: Our construct is a valuable tool to determine the direct targeting relationship between different signal transduction pathways and CHD7 gene expression. We believe that this work will be important for better understanding of the molecular mechanisms that lead to greater CHD7 expression in brain tumor tissue.

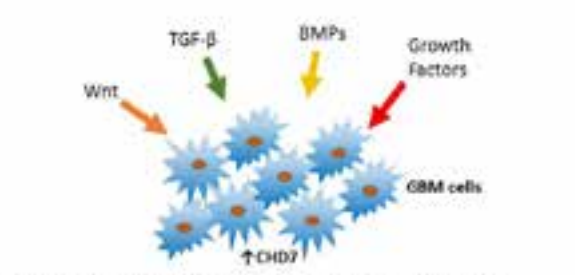

Figure: Deregulated signaling pathways in Glioblastoma (GBM) that could contribute to increased CHD7 expression in tumor cells.

Biography

Raquel Arminda Carvalho Machado has her expertise in Neural stem cells and neural plasticity. During her Master degree in Cell Biology Department of the ETH Zurich, she focused on "Reprogramming adult hippocampal neural stem/progenitor cells and their potential remyelination capacity", supervised by Prof. Dr. Sebastian Jessberger. Her current work integrates this knowledge applied to the oncology field. In the last years, she has been particularly interested in the function of CHD7 in glioblastoma. The construction of a CHD7 promoter luciferase reporter vector is an important step of this work which will certainly contribute to a better understanding of the role of the tumor microenvironment in the modulation of CHD7 expression in GBM cells.

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Bengt Åke Andersson et al., J Clin Epigenet 2017, 3:4
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Effect of cigarette smoking on circulating plasma miRNA in healthy individuals

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[2]Linköping University, Sweden
[3]Institute for Postgraduate Dental Education, Jönköping, Sweden

Introduction: Cigarette smoke contains toxic, carcinogenic, reactive and oxidant substances that causes DNA damage, transform epithelial cells phenotype and genotype, induce inflammation and remodeling immune response. Micro RNAs (miRNAs) are non-coding RNAs involved in gene expression in multicellular organisms at epigenetically level. MiRNA influence cell cycle progression, inflammation and smoking related tumours. miRNA expression pattern is essential for correct differentiation of immune cells.

Materials & Methods: A total of 140 healthy individuals, 39 current cigarette smokers and 101 individuals with no current or previous history of tobacco use (non-tobacco users) were included in this investigation. A panel of 11 miRNA was chosen in this study based on their documented associations in inflammation, immune response, oncogene regulation, tumor suppressor genes and they were all expressed in analysed plasma. Extraction and quantification of miRNA from plasma, miRCURY LNA™ Universal microRNA PCR system (Exiqon, Denmark) were used.

Result & Discussion: Of the 11-analysed miRNA, only miR-21-5p showed statistically significant decreased levels in 39 smokers compared to 101 non-tobacco users. miR-21 has been described as an oncomir and increased levels of miR-21 have been seen in several types of solid tumours and haematological malignancies. The epigenetic alterations of miRNA in smoking related head and neck cancer will be evaluated in further studies.

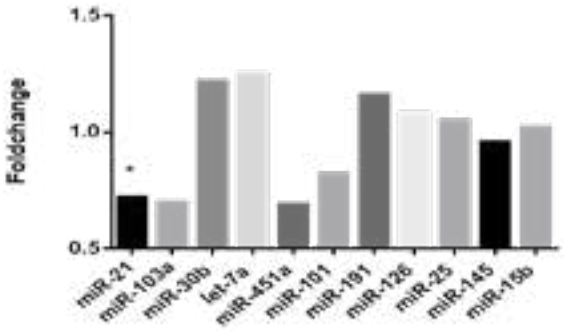

*Figure. Fold change between smokers and non-smokers on levels of miRNA . Adjustment was done for age and gender. *Statistically significant levels between smokers and non-smokers.*

Biography

Bengt Åke Anderson's research projects focusing on diagnostic and prognostic biomarkers associated with the effect of cigarette smoking on human health, cancer risk and clinical outcome of smoking related cancer.

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Notes:

Nesreen Mohamed Sabry Afifi Mattar, J Clin Epigenet 2017, 3:4
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Preliminary results of continuation maintenance treatment with pemetrexed in advanced non-squamous non-small cell lung cancer (NSCLC) patients after prior induction chemotherapy– single-arm phase II study

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Statement of the Problem: Lung cancer remains one of the leading causes of cancer-related death worldwide. Extending the duration of treatment with the initial platinum based chemotherapy beyond four to six cycles has been evaluated. The aim of this study is to investigate efficacy and toxicity of continuation maintenance treatment with pemetrexed (Alimta) in patients displaying disease control after four cycles of induction with cisplatin plus pemetrexed in advanced non-squamous (NSCLC).

Methodology & Theoretical Orientation: Between April 2013 and April 2015, 16 patients with pathologically proven stage III/IV, non-squamous NSCLC, in Clinical Oncology Department, Tanta University Hospital and Tanta Insurance Hospital who had received prior four cycles of induction with cisplatin (75 mg/m2) plus pemetrexed (500 mg/m2) every 21 days without disease progression were enrolled. Patients received continuation maintenance treatment with pemetrexed (500 mg/m2, every 21 days). The primary endpoints of the study were the overall survival and progression-free survival and the secondary end point was the safety profile.

Finding: A total of 64 chemotherapy cycles of continuation maintenance pemetrexed were administered. Patients were treated with a median number of 4 cycles (range 2-30 cycles). Two patients received no more than 2 cycles due to rapid disease progression. The estimated median PFS and OS were 7.5 and 17 months, respectively. Treatment-related adverse events were manageable with only 1 patient (6.25%) suffered from Grade 3 anemia and another 1 patient (6.25%) suffered from Grade 4 neutropenia. All patients received full doses of pemetrexed throughout the study. There was no treatment-related death.

Conclusion & Significance: Using the continuation maintenance regimen with pemetrexed preceded by four cycles of induction with cisplatin plus pemetrexed represents an obvious treatment advance with an acceptable clinical profile for patients with non-squamous NSCLC.

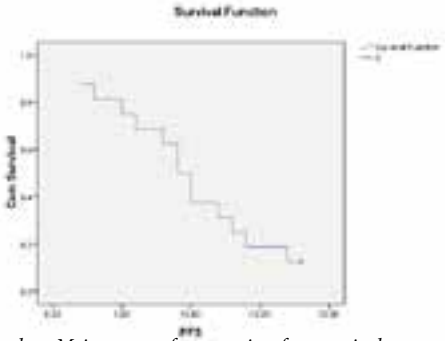

Figure 1: Kaplan–Meier curve of progression-free survival.

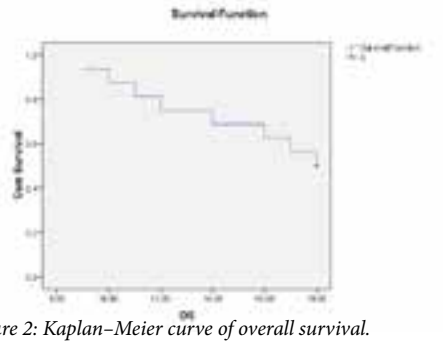

Figure 2: Kaplan–Meier curve of overall survival.

Biography

Nesreen Mohamed Sabry Afifi Mattar works as a Lecturer of Clinical Oncology at Tanta University Hospital and as a Consultant in Insurance Hospital. He has experience in Teaching to the Postgraduates. He completed his Master Degree in comparative study between chemoradiation and surgery in bladder cancer. His MD was about comparative study between R-CHOP and CHOP in DLBC NHL according to biomarker mutation (bcl2, p53). Recently, he has published a paper about the role of lapatinib in combination with letrozole in postmenopausal breast cancer women.

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Neimat Abd El Hakam Yassin, J Clin Epigenet 2017, 3:4
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Comparison of the anticancer effects of the histone deacetylase inhibitor panobinostat with doxorubicin on pre-clinical models of hepatocellular carcinoma

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Background & Aim: Panobinostat is the most recent histone deacetylase inhibitor approved for treatment of relapsed and recurrent multiple myeloma. In this study, we compare its effects with doxorubicin which is already used for trans-arterial chemoembolization of hepatocellular carcinoma (HCC).

Methods: Anti-cancer effects of panobinostat and doxorubicin are tested in DENA induced HCC in rats by pathological examination of liver sections and measuring liver enzymes. Anti-proliferative and pro-apoptotic effects are tested by measuring cell viability, P53, pd1 expression and cell cycle analysis in HepG2 cell line cultured with both drugs.

Results: Panobinostat was found to highly significantly inhibit Heppar-1 and VEGF levels and both drugs decrease ALT levels while AST was increased in panobinostat groups. Panobinostat was found to highly significantly induce apoptosis and decrease cell proliferation more than doxorubicin.

Conclusion: These results suggest that panobinostat may be a potent alternative to doxorubicin for treatment of HCC.

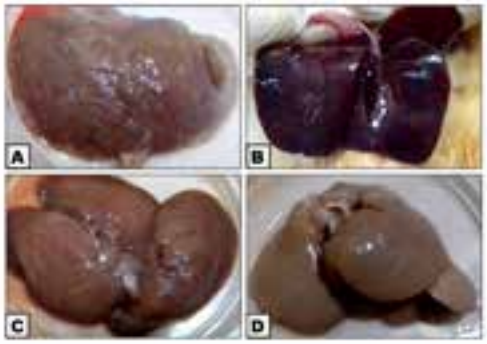

Figure 1 A&B: Liver of rats in DENA group. C: Liver of rat treated with intraportal panobinostat. D: Liver of rat treated with doxorubicin.

Biography

Neimat Abd El Hakam Yassin completed her PhD on experimental use of panobinostat in hepatocellular carcinoma at Clinical Pharmacology Mansoura, Faculty of Medicine. She is an Assistant Lecturer and has experience in teaching and research of pharmacology since 2007 in Clinical Pharmacology department in Faculty of Medicine, Mansoura University. She has a Master Degree in the use of hormonal antagonists in tumor therapy.

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Investigating the role of nitric oxide on DNA methylation in breast cancer cells

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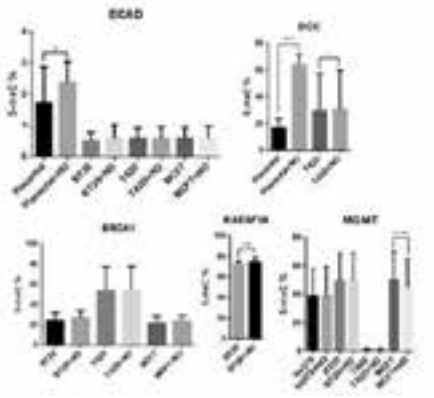
Introduction & Aim: Breast cancer is a predominant neoplastic disease among women and regardless of the disease subtypes it has been proposed that genetic mutations and epigenetic alterations caused by environmental factors may affect tumor development and growth. Nitric Oxide (NO), a free radical, is a well-known antioxidant has various roles in normal physiology. However, NO is also an important element in tumor microenvironment and has been linked to tumor growth. NO production is elevated in various human tumors including breast cancer. NO dependent gene regulation and histone methylation has been shown in cancer cells. As NO promoted deamination of 5-meC, it can be hypothesized that NO exposure can induce C-T transition in cancer cells. To exploit this hypothesis, we aimed to evaluate gene promoter methylation of BT-20, T42D and MCF-7 cell lines upon NO treatment.

Materials & Methods: Placental cell line was used for normal cell control. E-Cadherin (ECAD), Deleted in Colon Cancer (DCC), Breast Cancer 1 (BRCA1), Secreted Frizzled Related Protein 1 (SFRP1), Ras-association domain family 1 (RASSF1A), O-6-Methylguanine-DNA Methyltransferase (MGMT) promoter methylations were assessed before and upon no treatment using ion torrent next-generation sequencing system after bi-sulfite conversion. Promoter methylation was determined as percentage of cytosine reads of the total cytosine and thymine reads of each CpG site. To compare differences between groups, student t-test was applied.

Results: In control cell line, the effect of NO on DNA methylation was evaluated only for ECAD and DCC genes as the reading counts were below 100 for other genes. We found that NO exposure increased promoter methylation percentage of ECAD and DCC genes in placental cells ($p<0.05$). However, no significant change was seen on other cell lines for ECAD. DCC gene promoter methylation was found higher in T42D cells compared to placental cells and the methylation was increased upon NO exposure in both cell lines ($p<0.05$). We didn't find any significant chance in BRCA1 gene promoter methylation upon NO treatment in all cell lines. RASSF1A gene methylation in BT-20 cells and SFRP1 gene methylation in T42D cells were significantly increased upon NO treatment ($p<0.05$).

Conclusion: Our results can be further expanded using different cancer cell lines and interpreting the gene expression levels. We believe our results will contribute to the studies to further investigate the role of NO in regulation of gene expression in cancer cells.



Biography

Berna Demircan Tan has completed her PhD degree in Biochemistry. She completed her Postdoctoral training in USA in 2006-2010. Her research efforts have focused on the epigenetic basis of cancer, particularly DNA methylation. She has publications and book chapters on her research field. Currently, she is working as an Associate Professor at Istanbul Medeniyet University, Istanbul, Turkey.

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Accepted Abstracts

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Gamma-radiation induced DNA methylation and gene expression alterations in human cancer cell lines – an epigenetic connection

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Background: Ionizing radiation induces cellular damages through both direct and indirect mechanisms, which may include effects from epigenetic changes. DNA methylation is a fundamental epigenetic event associated with tumor progression. Hypermethylation of CpG islands at active gene promoters leads to transcriptional repression, whereas hypo-methylation is associated with gene over expression.

Objectives: To identify relative sensitivity and resistance to γ-radiation and to determine the effect of γ-radiation on gene-specific methylation pattern in human tumor cell lines that may be associated with altered gene expression.

Methods: Effect of radiation on gene-specific methylation as well as expression changes were identified using microarray hybridization analysis in radio-resistant and radio-sensitive cell lines which was further validated by bisulfide genomic sequencing. Systems biology approach was used to identify the activation of several novel signaling pathways which are silenced by DNA methylation.

Results: Our study demonstrates that γ-radiation alters DNA methylation at gene-specific level and differential expression leading to transcriptional activation of genes which are silenced by epigenetic mechanisms.

Conclusion: These results provide important information on alterations in DNA methylation and possibly associated with altered gene expression due to radiation effects and may reveal correlations between responses and either diagnosis or prognosis, and such *in vitro* validation marks an important step in the development of potentially informative radiation exposure biomarkers.

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Targeting histones and HDACs to bypass resistance mechanisms in gastric cancers: Relevance for immunogenic cell death and immunotherapy

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Gastric cancer remains a health issue in European countries with an average survival of 10 months due to a late diagnosis and the low efficiency of the standard therapies (e.g. cisplatin) on advanced cancers. One of the causes of cisplatin inefficiency is the high rate of mutation in the tumor suppressor gene p53. This emphasizes the necessity to develop innovative therapeutic strategies. To this end, we investigate the response to cisplatin using whole genome, mi-Rome, and proteome analyses to identify key altered signaling pathways. We identified several epigenetic regulators, including HDACs. Based on these findings, in collaboration with chemists, we developed several strategies to target histones and HDACs to treat gastric cancers. We identified a ruthenium complex that interacts directly with histones and causes alterations in metabolic pathways (e.g. unfolded protein response pathway) to induce immunogenic cell death. This mode of action allows this drug to bypass resistance mechanisms of cisplatin, such as p53 mutations. In parallel, we analyzed the effects of combinatory treatment associating cisplatin with pan or selective HDAC inhibitors. We found that they cooperate to induce synergistic cytotoxicity on gastric cancer cells. Interestingly, the combination of HDAC inhibitors and cisplatin inhibits p53 expression by a transcriptional mechanism. Surprisingly, despite the down-regulation of p53 protein levels, the synergistic induction of apoptosis by the combinatory treatment is dependent upon p53. However, we show that the combination of drugs in cells with p53 mutation is still able to induce a synergistic cytotoxic effect independently of apoptosis but rather through autophagy cell death. Together our work proposed novel and innovative anticancer strategies for gastric cancer allowing to bypass resistance mechanism by targeting epigenetic regulators or histones. By inducing immunogenic cell death, these therapies may cooperate with the most recent immunotherapeutic approaches develop to treat cancers and alleviate some of their limitations.

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Methyl-CpG binding proteins: Guardians of the epigenome

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All members of the Methyl-CpG-binding domain (MBD) protein family, except for MBD3, have been described to bind with high affinity to single methyl-CpG di-nucleotides, thereby silencing gene expression and dampening transcriptional noise of highly methylated, repetitive elements. In contrast, Ten-Eleven-Translocation (TET) proteins were shown to catalyze the conversion of 5 mC to 5 hmC, 5 fC and 5 caC in an iterative, Fe(II)-and oxoglutarate-dependent oxidation reaction, which is followed by the erasure of the repressing epigenetic mark. In this context, we aimed to elucidate the interplay of the MBD protein family and the recently described TET-mediated, active demethylation process. To this end, we quantified and compared global levels of 5 mC and its derivatives, transcriptional level, genomic stability and chromatin structure in human and murine cells as physiological consequences of 5 mC elimination. Moreover, we extended these analyses to the loss of function of the X-linked MECP2 gene, which causes Rett syndrome, a debilitating neurological disorder. We show that Mecp2 and Mbd2 protect 5 mC from Tet1-mediated oxidation in a concentration dependent manner *in vivo* and *in vitro*. The protection mechanism is not based on competition for 5 mC per se but rather on sequence unspecific coverage of DNA and correlates with the respective MBD protein dwell time on DNA. As a biological consequence, we measured increased 5 hmC level in neurons of a mouse model for Rett syndrome with concomitant reactivation of epigenetically silenced peri-centric DNA repeats.

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Profiling epigenomic landscapes and gene regulatory networks

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Active Motif, USA

Chromatin immunoprecipitation followed by next generation sequencing (ChIP-Seq) and other genome wide technologies have been integral in advancing our understanding of how epigenetic phenomena are regulated and how they affect gene expression. However, as we ask more complex questions the limitations of traditional genome-scale approaches have motivated researchers to develop new and improved methodologies for the characterization of the epigenomic landscape. This presentation will cover some of the progress that we and others have made in advancing the traditional ChIP-based assays and will include a new ChIP-Seq spike-in method for improved normalization and quantitation, a unique engineered DNA-binding molecule-mediated ChIP technology using the CRISPR system for dissecting the chromatin structure of your genomic regions of interest, and the development of a novel transposase based ChIP assay in which each antibody is conjugated to a barcoded transposome enabling the investigation of multiple targets within the same sample. These advanced technologies can be applied in new fields and diverse models to gain a deeper understanding of the complex regulatory mechanisms governing our genomes.

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SOX18 transcription factor interactome: Protein-protein interaction a new road for anti-cancer drug discovery SOX18 transcription factor interactome: Protein-protein interaction a new road for anti-cancer drug discovery

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Pharmacological targeting of transcription factors holds great promise for the development of new therapeutics, but the strategies based on blockading DNA binding, nuclear shuttling, or individual protein partner recruitment have had limited success to date. A single transcription factor has multiple transcriptional effects that are context-dependent. This versatility of activity is thought to be mediated by different protein-protein interactions (PPIs). Therefore, these PPIs offer a new avenue for the selective pharmacological modulation of transcription factor activity, which will lead to develop novel therapeutics. Transcription factors typically engage in complex interaction networks, likely masking the effects of specifically inhibiting single protein-protein interactions. Here, we used a combination of genomic, proteomic and biophysical methods to discover a suite of protein-protein interactions involving the SOX18 transcription factor, a known regulator of vascular development and thus cancer metastasis. We describe a small-molecule that is able to disrupt a discrete subset of SOX18-dependent interactions. This compound selectively suppressed SOX18 transcriptional outputs *in vitro* and interfered with vascular development in zebrafish larvae. In a mouse pre-clinical model of breast cancer, treatment with this inhibitor significantly improved survival by reducing tumor vascular density and metastatic spread.

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Energy healing yoga and energy healing treatments with human energy stations

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Humans who have program beliefs which limit their experience for a higher capacity for love, optimal health, joy and ease on Earth. As an expert in movement, author has witnessed and become aware of the unique experience that humans have when engaging in the practice of yoga, somatic movement and guided meditation-whereas they literally witness their own illusion of physical, mental or emotional limitations and witness a new perspective and possibility every time they learn a new healing pose or mental/emotional which at one time, perhaps even at the beginning of that class, they thought they could not achieve. Upon guiding thousands of classes in populations varying from homeless to mega corporate campuses and from ages 1-day old to 93, it became apparent that these movement and visual practices were creating opportunity for belief-change work taking place in yoga classes, therefore, concluding that epigenetics were indeed a part of yoga, meditation and somatic movement. As further multiple practices of energy healing such as Theta Healing, Reiki and a variety of others were being practiced with individuals, these energies organically became infused into all group classes, and the effects are profound. Companies who were once interested in more of a physical fitness noontime yoga class became more interested in restorative yoga which transitioned into energy healing yoga which at one time sounded weird, eight-years into these practices on campus because normal. Human energy station's desire is to educate humans on the true benefits of energy healing through movement, meditation and hands-on therapy and the importance of choosing it to be a normal and regular implementation to their self-care, which will create changes in our personal human experience.

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Epigenetic therapies and the development of personalized treatment of genetic pathologies

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Despite the investment of a substantial amount of technological, financial and academic resources, there has been minimal progress in the attempt to reduce the mortality rate associated with multitudinous forms of cancer and other genetic diseases. The advancement in the understanding of epigenetics and its impact on gene expression has introduced a potential gateway through which early detection of genetically influenced bio-pathologies can be experienced. Through the use of epigenetic data, it may be possible to detect or even anticipate the disruption of epigenetic networks that subsequently lead to the development of a number of major pathologies that include chromosomal instability syndromes, cancer and mental retardation. The introduction of epigenetics as a diagnostic tool may not only create the capacity to detect the aforementioned pathologies, it may also reveal other pathologies that are caused by epigenetic alterations. There is also great potential in the development of therapies that are centered in epigenetic processes that include DNA methyl transferase and histone deacetylases, as well as enzyme inhibitors, all of which have shown anti-tumorigenic promise as it pertains to certain malignancies. Epigenetics also offers great promise in the area of personalized treatment based on certain genetic predispositions. The study of epigenetics creates the opportunity to integrate advanced technologies and concepts into the process of diagnosis, prognosis and therapeutics — ultimately improving on the current dismal results being experienced.

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The role of imprint instability in human cancer

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The development of cancer in humans is not only caused by genetic lesions (mutations, deletions, translocations etc.) but also by epigenetic aberrations. One epigenetic phenomenon whose deregulation contributes to the development and progression of cancer in humans is imprinting, the parent-of-origin specific expression of genes. A causal role of imprinting aberrations in human carcinogenesis is suggested by several human disorders, e.g., complete parthenogenesis in ovarian teratomas and androgenic conception in hydatidiform moles. Contribution of imprinting defects in cancer is best exemplified in patients with Beckwith-Wiedemann Syndrome (BWS) which is associated with a high risk of cancer compared to the general population. Since some genes demonstrate developmental stage-specific or tissue specific imprinting, the study of imprinting can be complicated and the comparison of results from different studies might be misleading. The use of proper controls for the identification of imprint alterations is of uppermost importance. These experimental challenges might be the reason why much less is known about imprinting defects in human cancer compared to aberrant promoter hypermethylation of tumor suppressor genes. Based on published information about validated imprinted genes, genome-wide expression and DNA methylation data, and results obtained with primary human patient samples (and not cell lines or animal models!), we started to identify deregulation of imprinted genes in breast and liver cancer and study the functional relevance of these findings. As documented in several publications from our group, we could show that deregulation of imprinted loci is an underappreciated but widespread phenomenon with clinical relevance. We could also show difficulty to identify switches in allelic expression are much more frequent than thought before and that deregulation of the only quite recently identified imprinting of the well-known tumor suppressor gene RB1 is a frequent event in liver cancer.

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DNA dysmethylation of various genes contributes to disease risk in progressive supranuclear palsy

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Statement of the Problem: Progressive Supranuclear Palsy (PSP) is a neurodegenerative tauopathy. The etiology of this complex disorder is poorly understood. An accumulation of multiple environmental, genetic, and possibly epigenetic factors is thought to eventually cause disease. Thus, advanced age contributes to risk as do certain polymorphisms of the genes MAPT, STX6, EIF2AK3, and MOBP. A possible role of epigenetic changes in PSP is currently under investigation and dys-regulation of several miRNAs has been reported. This study was performed to investigate whether DNA dys-methylation might also contribute to disease.

Methods: DNA was extracted from forebrains of 94 PSP patients (age at death 72±5.3 years) and 72 controls (76±7.9 years). Methylation was studied using the Infinium 450k array of Illumina that includes more than 485,000 potentially methylated CpG sites distributed over the entire genome. 200 ng of bisulfite-converted DNA were hybridized to the array. Arrays were scanned and GenomeStudio software (version 2011.1; Illumina Inc., San Diego, CA) was used to measure the intensity of DNA methylation signals on the arrays. DAVID Bioinformatics resources 6.7 were used for functional analysis of hyper- and hypo-methylated genes. Highly significant ($P<0.01$) methylation differences of >1% were compiled and the location of these differentially methylated sites was analyzed. Functional annotation clustering was performed and enrichment scores >1.3 were considered significant.

Findings: Significant ($p<0.01$) methylation differences of >1% were detected at 621 sites amounting to 383 protein-coding genes. At high stringency ($p<0.01$, methylation difference >5%), dys-methylated CpGs were found associated with 30 genes. 142 of the dysmethylated sites were also detected in age- and gender-matched cohorts (difference >1%, $p<0.01$). Disease-specific changes were found at 59 and age-dependent methylation differences were detected at 16 CpG sites. Of the genes dys-methylated by >5% ($p<0.01$) differences were disease-specific in 8 and age-dependent in 3 genes. While dysmethylation of >5% affected one or a few CpG sites in most genes, in one, i.e. DLX1 hyper-methylation was found at multiple sites including a CpG island in the 3´-untranslated region (UTR) of the gene. This and flanking genes (DLX2, METAP1D) are methylated in an age-dependent manner. Among the disease-specifically dys-methylated genes, HDAC4, which might serve as a therapeutic target, was hypo-methylated. Conclusion: The data suggest that both disease-specific and age-dependent, i.e. premature dysmethylation of various genes contribute to PSP.

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Therapy stratification of rectal cancer patients by epigenetic testing

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Considerable progresses have been made in the development of neoadjuvant colorectal cancer (CRC) therapeutics and many patients respond to neoadjuvant chemo-radiotherapy resulting in tumor down staging. In up to 20% a complete pathological response is observed, characterized by absence of residual primary tumor in the pathological specimen that translates into excellent disease-free survival of more than 90%. For such patients, an observational watch-and-wait approach, may replace surgical intervention. However, therapeutics are still away from optimal and the main obstacles in patient treatment are moderate efficacy and intrinsic resistance. In addition, a complete response to neoadjuvant chemo-radiotherapy can currently be determined only by a pathological evaluation following surgery. This highlights the need for predictive biomarkers allowing stratification of patients according to their response to neoadjuvant chemo-radiotherapy. Epigenetic marks, like DNA methylation have been identified as potential candidate biomarkers for the prediction of treatment efficacy. Therefore, we investigated the role of DNA methylation in therapy response to identify a DNA methylation signature, able to stratify CRC patients in therapy responders and non-responders to advice clinicians whether one can stick to a wait-and-watch approach, or to surgery. A retrospective epigenome-wide-DNA-methylation study using 48 FFPE CRC samples was conducted using Illumina's Human Methylation EPIC Bead Chip. Biostatistics confirmed distinct DNA methylation profiles for patients in accordance to their response to neoadjuvant therapy with an almost perfect discrimination between the groups. The most striking observation to emerge from the data was the gradual character (either increasing or decreasing) of the methylation status from non-responder, via partial responder towards full responder to neoadjuvant therapy. Consequently, the identified DNA methylation signature has predictive potential to shed light on patient's individual response status to neoadjuvant chemo-radiotherapy and can be used for therapy decisions whether a watch-and-wait approach or operative intervention is the more appropriate strategy.

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A translational approach for epigenetic regulation of tumor suppressor genes and microRNAs in malignant plural mesothelioma (MPM)

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Malignant Plural Mesothelioma (MPM) is an aggressive cancer caused by asbestos explore. Due to heavy use of asbestos as building material in the pass and long latency of MPM, predict the number of cases will max out during the 2020s and 2030s. Despite the combination of cisplatin/gemcitabine treatment, there is currently no treatment option for MPM with median survival of 9-12 months. Treatment options for MPM are mainly palliative in nature as most patients will be confined with recurrence of the disease and resistance of chemotherapy. It is urgently needed to discover treatment options for MPM. Using microRNA microarray study, we found down-regulation of microRNA is a common event in MPM. The re-introduction of potential tumor suppressor microRNA in MPM led to suppression of tumor cell growth *in vitro* and *in vivo*. Our contribution of microRNA study led to the world's first clinical trial of microRNA replacement in MPM. We have further discovered the down regulation of microRNAs are a result of DNA hypermethylation of their host gene promoter region. We have planned multidiscipline studies including epigenetic regulation in MPM to understand the fundamental biology of MPM in discovering newer treatment options. Dr. Cheng is currently the lead guest editor of the special issue "Epigenetic Biomarkers in Cancer" to recruit high standard research publications in these areas which ultimately may contribute to newer diagnostic tools for MPM.