Here we use a non-invasive method to measure the metabolic phenotype of single colon cancer cells *in vivo*. By using NADH as optical biomarker and the phasor approach to Fluorescence Lifetime microscopy (FLIM) we identify cancer metabolism related to different rates of glycolysis, cell growth and proliferation of cells. We perform label-free Phasor FLIM on living and actively-perfused xenograft tumors. Colon cancer cells are injected subcutaneously into immune deficient (NSG) mice and tumors are allowed to grow for three weeks. The tumor vasculature is labeled by injecting TRITC Dextran into the tail vein and xenografts are exposed via a skin flap, still perfused by their feeder vessels.

FLIM distinguishes collagen fibers, the tumor stroma, adipocytes, blood vessels and single cancer cells within the living tumor. By measuring NADH lifetime, we quantify the relative concentration of free and bound NADH in single cancer cells, which reflects the cellular redox NADH/NAD+ ratio and balance of oxidative phosphorylation and glycolysis. We investigate the tumor microenvironment by characterizing the distribution of the metabolic fingerprint of single colon cancer cells and by mapping the three-dimensional metabolic heterogeneity of the tumor at different distances from blood vessels.

Our method permits a non invasive measurement of single cancer cell metabolism in a living intact tumor microenvironment. It allows monitoring of spatial and temporal dynamic changes of tumor metabolism upon different physiological conditions such as blood flow, tissue oxygenation levels, nutrient availability and drug delivery.

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Quantitative Intra-Vital FRET Imaging Reveals Enhanced Sympathetic Adrenergic Tone in Femoral Arteries in Chronic Angiotensin Ii-Infused Biosensor Mice

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Hypertension only exists in living animals. Administration of Angiotensin II (Ang II) to animals increases blood pressure. One of the major effects of Ang II is to cause vasoconstriction via directly activating the Ang II type-1 receptor (AT_1R) in blood vessels. The aim of this study is to determine whether or not chronic Ang II infusion evokes conduit arterial constriction *in vivo*, and if so what the underlying mechanisms are. Ang II (400 ng/kg/min) or vehicle (saline) was continuously infused for 2 weeks by subcutaneously implanted osmotic mini-pumps in exogenous myosin light chain kinase FRET biosensor mice. Blood pressure, measured with an intra-carotid artery catheter under isoflurane anesthesia, was significantly elevated in Ang II-infused mice. Mid-thigh femoral arteries were slightly dissected and superfused with physiological saline solution. Simultaneous *in vivo* arterial diameter and quantitative cytoplasmic [Ca^{2+}] in smooth muscle cells within intact arteries were measured. Under resting condition, femoral arteries from Ang II-infused mice exhibited significantly increased basal tone and cytoplasmic [Ca^{2+}]. Local application, in the superfusion solution, of phentolamine (1 μM), a non-selective α adrenoceptor antagonist, and prazosin (100 nM), a selective α1 adrenoceptor antagonist, caused significantly more relaxation and [Ca^{2+}] reduction in Ang II than in vehicle group. Dose-dependent vasoconstriction induced by an α adrenoceptor agonist, phenylephrine, however, was similar, suggesting a lack of expression or sensitivity change of the local adrenoceptor. In contrast, acute Ang II-induced vasoconstriction was reduced in chronic Ang II-infused mice, suggesting a possible down-regulation of AT_1R. These results indicate that, in chronic Ang II-infused mice, albeit the reduced acute vasoconstrictor response to Ang II, there is an increased sympathetic adrenergic tone of femoral arteries, which correlates with blood pressure elevation. (AHA SDG0735461N, NIH R01 HL107654).

EPR Spectroscopy

1758-Pos Board B650

Dynamics of Histone Tails within Chromatin

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The physical and material properties of chromatin appear to influence gene expression by altering the accessibility of proteins to the DNA. The fundamental unit of chromatin, the nucleosome, consists of DNA wrapped around a core histone octamer protein. The tails of the histone proteins are flexible domains that are thought to play a role in regulating DNA accessibility and compaction; however the molecular mechanisms for these are not understood. using Site Directed Spin Labeling and Electron Paramagnetic Resonance (CW-EPR), we probe the dynamics of the histone tails within chromatin. The goal of this study is to further understand the function of histone tails and the part they play in gene regulation.

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Sequence-Dependent Local Environment in P53 Response Element Probed by Site-Directed Spin Labeling

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The p53 response elements (RE) are a family of DNAs that are specifically recognized by the tumor suppressor protein p53. Binding of p53 to REs leads to transcription activation or suppression of a diverse set of genes that regulate a number of key biological processes. The molecular basis of p53-RE interaction has been a topic of intensive studies, however, it remains unclear how this one p53 protein can specifically recognize a large number of DNA targets in order to elicit diverse functions. As sequence dependent properties of DNAs are a key determinant in protein-DNA interaction, information on local structure and dynamic features in p53 RE is important in deciphering p53-RE recognition. Towards this goal, we report results on probing p53-RE using site-directed spin labeling, where a site-specifically attached nitroxide radical is monitored to obtain structural and dynamic information of the parent macromolecule. A nucleotide-independent labeling scheme is used to scan the nitroxide along RE sequences, and continuous-wave electron paramagnetic resonance spectroscopy is used to monitor the rotational motion of the nitroxide, which encodes DNA structural and dynamic properties at the labeling site. Spectral variations have been observed, with analyses of which reveals sequence-specific local environment within the RE. This will aid in understanding p53 recognition of target DNAs.

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Incorporation of a Rigid TOAC Spin-Label as a Non-Native Amino Acid into a Full-Length Protein by In Vitro Translation using Amber Codon Suppression

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The development of EPR site-directed spin-labeling (SDSL) techniques has provided a powerful avenue for the study of membrane protein structure and dynamics. One of the advantages of EPR is the ability to obtain distance and dynamic information about particular protein regions. The accuracy of this information is highly dependent on the spin-label that is used. Labels with less inherent mobility are better reporters of protein backbone dynamics and give more sharply defined distance parameters. TOAC is a commonly used rigid spin-label that can be integrated into the protein backbone as an amino acid analog. However, its current use is limited to the study of small peptides that can be only generated synthetically (solid-phase peptide synthesis). A new biochemical approach for incorporation of non-natural amino acids during translation using a synthetically loaded amber suppressor tRNA has successfully generated TOAC-labeled protein but not in sufficient yields for EPR studies. Here, we describe the use of an amber codon suppressor tRNA and a continuous exchange cell-free (CECF) expression system for in vitro translation of a small thylakoid membrane protein, Tha4, containing site-specific integration of TOAC. Further, we demonstrate successful incorporation of the TOAC-containing Tha4 into liposomes by supplying POPC vesicles during the translation reaction. By combining new techniques in suppressor tRNA and continuous exchange cell-free (CECF) translation, we have developed a method for translation of full length proteins with site-directed insertion of non-natural amino acid spin labels in yields applicable to CW-EPR experiments.

1761-Pos Board B653

Probing the Secondary Structure of Membrane Proteins with the Pulsed EPR ESEEM Technique

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A novel approach is being developed to probe the secondary structure of membrane proteins and peptides qualitatively utilizing the three-pulse Electron Spin Echo Envelope Modulation (ESEEM) pulse sequence. The α-helical M2delta subunit of the acetylcholine receptor incorporated into phospholipids bicelles has been used as a model peptide. In order to demonstrate the practicality of this method, a cysteine mutated nitroxide spin label is positioned 1, 2, 3 and 4 residues away from two types of deuterated Leu side chains (denoted *i+1* to *i+4*).

The characteristic periodicity of the α-helical structure gives rise to a unique pattern in the ESEEM spectra. So far, this method has been successful with ^2H-labeled Val and Leu. The deuterium modulation depth is significantly