binding to mitochondria inhibits mPTP. To better understand the mechanism(s) behind the protective effects of HKs, we overexpressed full-length HKI and HKII (FL-HKI and FL-HKII, respectively), their truncated proteins lacking the N-terminal hydrophobic domains (Tr-HKI and Tr-HKII, respectively), and catalytically inactive proteins (Mut-HKI and mut-HKII, respectively) in human embryonic kidney (HEK293) cells. The truncated enzymes cannot bind to mitochondria but can phosphorylate glucose, whereas the catalytically inactive enzymes can bind to the mitochondria but do not phosphorylate glucose. The cells overexpressing these constructs were subjected to oxidant stress followed by measurement of mitochondrial membrane potential and cell death. Overexpression of FL-HKI and FL-HKII resulted in complete protection against oxidant-induced loss of mitochondrial membrane potential and cell death (survival percentage of 96 ± 9 and 95 ± 5 for FL-HKI and FL-HKII, respectively). Although overexpression of the truncated and mutant proteins reduced cell death, the degree of protection was about 40 to 50% less than that of the full-length proteins. Furthermore, FL-HKI and FL-HKII inhibited mitochondrial permeability transition (MPT) in the presence of H_2O_2, whereas the truncated and mutant forms only caused partial inhibition. Similar results were obtained when these proteins were expressed in primary neonatal rat cardiomyocytes using an adenoviral technique. To understand the mechanism for the protective effects of HKs, we measured VDAC phosphorylation in cells overexpressing these proteins. Overexpression of FL-HKI and FL-HKII resulted in a 5- to 10-fold increase in VDAC phosphorylation. The mechanism for VDAC phosphorylation appears to be through PKC-ε as inhibitors of this enzyme led to a reversal of this process. These results suggest that both glucose phosphorylation and inhibition of mPTP contribute to the protective effects of HKI and HKII. Furthermore, overexpression of HKI and HKII leads to VDAC phosphorylation in a PKC-ε-dependent pathway. These findings bear implications of HK overexpression and binding to the mitochondria as a potential clinical treatment strategy for various forms of human disease.

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ASBESTOS-INDUCED INFLAMMATION AND FIBROSIS ARE REGULATED BY RAC1. L.A. Tephly, A. Dodd, P.S. Thorne, M. Glogauer, A. Carter, University of Iowa Carver College of Medicine, Iowa City, IA; Toronto, ON, Canada.

The release of TNF-α by alveolar macrophages plays an integral role in the pathogenesis of the inflammatory response that occurs in the lungs of patients with asbestosis. In contrast to most stimuli, our data demonstrate that the p38 MAP kinase is a positive regulator and the ERK MAP kinase is a negative regulator of TNF-α production in response to asbestos stimulation. However, limited data are available on the upstream signaling pathways linking asbestos with TNF-α expression. The GTPase Rac1 is an upstream second messenger that plays an important role in inflammation. We found that TNF-α production is augmented in monocytes overexpressing Rac1. We hypothesized that Rac1 plays a pivotal role in differentially modulating MAP kinase activation and that this differential activation is critical for TNF-α gene expression in human monocytes stimulated with asbestos. We explored the role of Rac1 in regulating the activation of MAP kinases. Our data demonstrate that overexpression of Rac1 increased p38 activity and abolished ERK activity. In contrast, overexpression of a dominant negative Rac1 increased ERK and inhibited p38. To determine if Rac1 and differential MAP kinase activity had any biologic significance, we exposed wild-type and Rac1 null mice to asbestos. Wild-type mice had significantly more inflammatory cell infiltration and produced more TNF-α compared with Rac1 null mice. More importantly, wild-type mice developed interstitial fibrosis, whereas Rac1 null mice exposed to asbestos were no different from saline controls. In aggregate, these data suggest that Rac1 has a pivotal role in regulating MAP kinase activity and TNF-α gene expression in monocytes, and the absence of Rac1 in inflammatory cells is protective against the development of pulmonary fibrosis after asbestos exposure.

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FATTY ACIDS ACTIVATE NUCLEAR FACTOR κB AND INDUCE INTERLEUKIN-6 PRODUCTION FROM MOUSE 3T3-L1 PREADIPOCYTES AND ISOLATED HUMAN SUBCUTANEOUS STROMAL-VASCULAR CELLS. P. Tu, S. Wong, S. Bigornia, B. Corkey, C. Apovian, W. Guo, Boston University School of Medicine, Boston, MA.

Obesity markedly elevates circulating proinflammatory cytokines, which are found to be associated with multiple chronic diseases, such as cardiovascular diseases and type 2 diabetes mellitus. However, the underlying mechanisms for systemic inflammation in obese individuals are poorly understood. One unambiguous physiologic change from lean to obese individuals is a chronic elevation of circulating free fatty acids (FFAs), imparted owing to enlarged fat cell size and population as well as impaired antilipolytic actions of insulin. FFA has been shown to induce cytokine production in several cell types, including macrophages. Obesity is found to be associated with increased macrophage infiltration in adipose tissue, but the cause of such is still not conclusive. On the other hand, adipose tissue is naturally enriched with preadipoctyes. Striking similarity has been documented between preadipocytes and macrophage with even conversion between the two cell types under adequate conditions. Paradoxically, little is known about how FFA affects the function of preadipocytes, the cell type that is closely associated with adipocyte-derived FFA. In this study, we showed that FFA directly activates NF-κB, the master proinflammatory transcription factor and its downstream products IL-6 and TNF-α in clonal preadipocytes (3T3-L1) and in isolated human adipose stromal-vascular cells (SVCs). Inhibition of NF-κB using NF-κB-specific inhibitor (TLCK), proteasome inhibitor (MG132), and siRNA-mediated inhibition of NF-κB-p105 significantly attenuated FFA-stimulated IL-6 production in both 3T3-L1 and human SVAs ($p < .05$ for all). Taken together, our findings suggest that FFA may play an active role in adipose inflammation in obese individuals via regulation of function of preadipoctyes.

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MARINOBUFAGENIN INHIBITS PROLIFERATION VIA NA^+/K^+-ATPASE-DEPENDENT DOWN-REGULATION OF ERK AND JNK SIGNALING IN CHINESE HAMSTER OVARY CELLS. M.N. Uddin, D. Horvat, S.S. Glaser, L.B. McLean, J.B. Puschett, Texas A & M University Health Science Center College of Medicine/Scott & White Hospital, Temple, TX.

MBG is an endogenous mammalian cardiotonic steroid that is involved in the inhibition of the sodium pump Na^+/K^+-ATPase. Increased plasma levels of MBG are associated with hypertension, renal failure, and preeclampsia. In an animal model of preeclampsia, we have shown that MBG levels are elevated prior to the development of hypertension, indicating that it may play a key role in the pathogenesis of preeclampsia, which is characterized by shallow placentation. We recently demonstrated that MBG impairs both the proliferation and growth factor–induced migration of first-trimester cytotrophoblasts (CTB), cells critical for placental development. The intracellular signaling mechanisms regulating MBG-induced inhibition of Na^+/K^+-ATPase and the impairment of CTB differentiation are unknown. Activation of MAP kinase signaling (such as ERK1/2, p38 and Jnk) is known to play a critical role in the regulation of cellular proliferation and differentiation. Thus, the aim of our study was to begin to elucidate the mechanisms by which MBG inhibits proliferation via down-regulation of MAP kinase signaling. Chinese hamster ovary (CHO) cells were used to elucidate intracellular signaling mechanisms. CHO cells have previously been used to study Na^+/K^+-ATPase transport activities. The phosphorylation of ERK1/2, p38, and Jnk1/2 was evaluated by ELISA and by immunoblotting for phosphorylated and total proteins from CHO cells stimulated with MBG (10 and 100 nM) at 0-, 10-, 30-, and 60-minute time points. Apoptosis was evaluated by caspase 3/7 activation. MBG inhibited the proliferation of CHO cells, indicating a Na^+/K^+-ATPase-dependent mechanism involving intracellular Ca^{2+}. MBG stimulated a significant time-dependent decrease in the phosphorylation of both ERK1/2 and Jnk1/2 but not p38. In addition to inhibition of proliferation, MBG stimulated a significant increase in caspase 3/7, indicating the activation of apoptosis. We conclude that the MBG-induced impairment of CHO cell differentiation/proliferation occurs via a Na^+/K^+-ATPase-dependent down-regulation of ERK1/2 and Jnk1/2 activation and activation of apoptosis. Therapeutic targeting of the MBG signaling pathway may provide treatment paradigms for preeclampsia.

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REPLICATION OF DNA PALINDROMES AND CHROMOSOMAL INSTABILITY. I. Voineagu, K. Lobachev, S. Mirkin, Tufts University, Somerville, MA; Atlanta, GA.

Long DNA palindromes are known to be unstable in bacteria and have more recently been shown to induce chromosomal breaks in yeast. It has also been shown through sequence analysis of the human genome that palindromic ALU repeats (a type of primate-specific transposon) tend to be excluded from the human genome, and this tendency has been attributed to their ability to induce chromosomal breaks. Since stalled replication forks can potentialy lead to chromosomal breaks, we tested the hypothesis that palindromic sequences such as inverted ALU repeats block replication fork progression in vivo. We looked at DNA replication through closely spaced inverted ALU repeats with different degrees of sequence homology in *Escherichia coli*, *Saccharomyces cerevisiae*, and COS-1 monkey fibroblasts. Inverted ALU repeats were cloned into episomal vectors, and their effects on replication fork progression were assessed using two-dimensional gel electrophoresis. We showed that long inverted repeats can stall DNA replication in prokaryotes, lower eukaryotes, and primate cells. The intensity of replication stalling in eukaryotes is not as dramatic as in prokaryotes, most likely owing to the shorter length of Okazaki fragments that gives fewer opportunities for secondary structure formation on the lagging strand template. These results can explain the tendency toward excluson of inverted ALU repeats from the human genome and the occurrence of chromosomal breaks.

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CLINICAL UTILITY OF RADIOGRAPHIC TEXTURE ANALYSIS PERFORMED ON DENSITOMETRIC CALCANEAL IMAGES. T.J. Vokes, M. Chinander, A. Pham, J. Wilkie, M. Giger, University of Chicago, Chicago, IL.

Background: Currently, the major challenge in osteoporosis research and clinical practice is to improve the assessment of fracture risk. Bone fragility is determined by bone quantity measured as bone mineral density (BMD) and by bone quality or structure that cannot be easily assessed using currently available methods. Radiographic texture analysis (RTA) is an image-based noninvasive method of evaluating bone structure through computerized analysis of trabecular pattern of bone radiographs. We have applied RTA to calcaneal images obtained using a portable densitometer. RTA yields the following features: RMS (root mean square variation, a measure of the variability in the radiographic texture pattern, the relative difference in the contrast between light and dark areas with higher values corresponding to the strong rich trabecular pattern), and its directional measure, sdRMS (standard deviation of RMS), which is a measure of anisotropy of the trabecular pattern, with higher values corresponding to the more directional texture pattern, and Minkowski fractal (MINK), a measure of roughness-smoothness of the trabecular pattern. In this study of patients referred for BMD testing at the University of Chicago, we examined (1) which biologic factors influence RTA and (2) how well RTA differentiates subjects with high and low bone fragility. **Methods:** In 837 patients (age 19–95 years, 759 women), BMD was measured at the lumbar spine, proximal femur, and calcaneus; clinical risk factors were obtained using a questionnaire; and RTA was performed on densitometric heel images. Fragility was defined as the presence of vertebral fractures detected on VFA (vertebral fracture assessment - densitometric spine image). The association of RTA with biologic factors or with prevalent vertebral fractures was examined using regression analysis. **Results:** (1) Relationship between RTA and biologic factors: In a multivariate regression analysis, significant predictors of sdRMS were BMI ($p < .001$), gender ($p < .001$), age ($p = .001$), race ($p = .004$), and heel BMD ($p = .009$). For MINK, the only significant predictors were BMI ($p < .001$) and heel BMD ($p < .001$). (2) Utility of RTA in assessing fragility was examined in a subset of 333 postmenopausal women who had no secondary causes of and were not receiving therapy for osteoporosis. In a univariate logistic regression analysis with the presence of prevalent vertebral fractures as a binary outcome, the separations between 49 women with and 284 without vertebral fractures using RTA and using BMD measurement were similar ($p = .002$ and $p < .001$ for sdRMS and MINK, respectively; $p = .01$, $p = .003$, and $p < .001$ for spine, heel, and hip BMD, respectively). In a multivariate logistic regression, there was a significant association of vertebral fractures with RTA, BMD, and age with odds ratio (OR) of having a vertebral fracture of 2.0 for each decade increase in age ($p < .001$), OR = 1.8 for a 1-unit decrease in hip BMD T-score ($p = .003$), and OR = 1.7 for a 1 SD decrease in sdRMS ($p = .003$) or MINK ($p = .008$). **Conclusion:** These results suggest that RTA of heel images obtained using a portable densitometer characterizes bone properties not measured by BMD. Specifically, RTA provides assessment of fragility that is not captured by the currently used measurements such as BMD and clinical risk factors (age). Addition of RTA to BMD and clinical risk factors can improve the stratification of fracture risk. Further studies are needed to determine whether RTA would be useful in selecting patients for different forms of osteoporosis therapy.

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THE ANTITUMORIGENIC EFFECTS OF WNT 7A AND FRIZZLED 9 ARE MEDIATED THROUGH PEROXISOME PROLIFERATOR–ACTIVATED RECEPTOR γ–DEPENDENT ACTIVATION OF THE RECEPTOR TYROSINE KINASE INHIBITOR SPROUTY 4 IN NON–SMALL CELL LUNG CANCER. R.A. Winn, M. Tennis, M. Van Scoyk, J. Won Sohn, Denver VAMC, Denver, CO.

The Wnt genes encode a family of highly conserved, secreted, cysteine-rich proteins that were originally identified as key regulators of early development in *Drosophila* and as proto-oncogenes in mammals. Wnts have been implicated in many human cancers, including colon, breast, and melanoma, but have not been clearly established in lung tumorigenesis. Recent studies from our laboratory indicate that the