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GENE EXPRESSION PROFILES OF 4-HYDROXY-N-DESMETHYL-TAMOXIFEN (ENDOXIFEN)- AND 4-HYDROXY-TAMOXIFEN (4OHTAM)-TREATED HUMAN BREAST CANCER CELLS DETERMINED BY CDNA MICROARRAY ANALYSIS. Y. Lim, MD, PhD, L. Li, PhD, Z. Desta, PhD, J. M. Rae, PhD, D. A. Flockhart, MD, PhD, T. C. Skaar, PhD, Indiana University, University of Michigan, Indianapolis, IN.

Recently, we have shown that endoxifen and 4OHTAM are equipotent inhibitors of estrogen action, with respect to estrogen receptor (ER) binding, proliferation, and inhibition of pS2 and PR gene expression. Since estrogens and anti-estrogens have effects on the expression of many additional genes, we tested the effects of endoxifen and 4OHTAM on genome-wide profiling in ER-positive MCF-7 cell lines by cDNA microarray analysis using the Affymetrix HG-U133A GeneChip. Cells were treated for 24 hrs with vehicle, 17β-estradiol (E2) 10^{-10}M, 4OHTAM 10^{-7}M, endoxifen 10^{-7}M, E2+4OHTAM, E2+endoxifen, and E2+4OHTAM+endoxifen. E2 treatment resulted in up- or down-regulation of ~213 genes which showed greater than 2-fold changes from the vehicle-treated group. 4OHTAM and endoxifen changed the expression of 58 and 52 genes respectively when added to the E2 treatment. Of these genes, 49 (84.5%) and 46 (88.5%) were estrogen-regulated genes. 4OHTAM and endoxifen had overlapping effects on 32 genes, but also brought about distinct patterns of gene regulation (26 vs. 20 genes were changed by only 4OHTAM or endoxifen, resp.). Among the 32 genes coregulated by both 4OHTAM and endoxifen, there was a significant correlation between the fold-effects brought about by these two drugs (R^2=0.964, P<0.01). These data indicate that the majority of genes influenced by active tamoxifen metabolites are estrogen-sensitive genes, but that some effects of tamoxifen may be estrogen-independent.

PDI-A-8

THE EFFECT OF HOMOCYSTEINE ON THE PRODUCTION OF INFLAMMATORY CYTOKINES. Y. Asanuma, MD, PhD, A. Oeser, BSc, E. Stanley, A. Shintani, PhD, C. M. Stein, MD, St. Marianna University School of Medicine, Vanderbilt University School of Medicine, Kawasaki, Japan.

Elevated C-reactive protein (CRP), homocysteine and inflammatory cytokines such as interleukin-6 (IL-6) are associated with increased cardiovascular risk. CRP has direct inflammatory actions stimulating the production of IL-6 and monocyte chemoattactant protein-1, (MCP-1) in whole blood. Homocysteine increases the production of MCP-1 and IL-8 in endothelial cells *in vitro*, but whether it has direct inflammatory actions in an environment more representative of *in vivo* biology, is not known. Thus, we examined the hypothesis that homocysteine would stimulate the production of IL-6 and MCP-1 in whole blood. Blood was drawn from 15 healthy subjects and incubated with saline, 1μg/ml lipopolysaccaride (LPS), 50 (n=8) and 100 μM/L (n=6) DL-homocysteine or 5 μg/ml human recombinant CRP for 24 hours. Both CRP and LPS significantly increased the production of IL-6 and MCP-1 more than 4-fold (P<0.001) but homocysteine did not. Homocysteine had little effect on IL-6 (fold change at 24 hours 1.4±0.4 (SEM) vs. baseline, P=0.14) or MCP-1 (0.9±4.5, P=0.08) production. Thus, concentrations of homocysteine that had inflammatory effects *in vitro*, and are in the range that occur in patients with hyperhomocystinemia had no effect on IL-6 or MCP-1 production in whole blood. A possible explanation is that the whole blood environment provides antioxidant defenses not present in cell culture and may thus attenuate the inflammatory effects of homocysteine, thought to be mediated by free radicals.

PDI-A-9

COX-2 INHIBITORS AND CARBONIC ANHYDRASE ACTIVITY. J. F. Knudsen, MD, PhD, U. Carlsson, PhD, P. Hammarström, PhD, G. H. Sokol, MD, L. R. Cantilena, Jr., MD, PhD, Uniformed Services University of the Health Sciences, Bethesda, MD.

Purpose: To compare the carbonic anhydrase inhibition (CAI) profile of selective and non-selective COX-2 inhibitors with the prototypic CAI acetazolamide (ACZ). **Methods:** The CO2 hydration activity of human carbonic anhydrase II (hCAII) was determined by a colorimetric method. The enzyme concentration was 5nM. Stock solutions of the inhibitors were dissolved in either ethanol or DMSO. The fraction of inhibitory activity was calculated by comparing CO2 hydration activity of the enzyme in the absence and presence of the test compounds (tested blind to the study hypothesis). Duplicate determinations were performed. Commercially available compounds purchased at the pharmacy and excipient free compounds were used. **Results:** The IC50 (nM) values (from the most potent CAI to least) were: ACZ, 7.5; celecoxib, 410; valdecoxib, 24800; rofecoxib > 3X10 5 and diclofenac > 3X10 5. The structural basis underlying the differences in potency is in all likelihood the pharmacophore SO2NH2, the common molecular moiety of acetazolamide, celecoxib and valdecoxib. The sulfonamide motif is absent from rofecoxib and diclofenac. **Discussion:** We have demonstrated an unusual inhibition binding profile for the COX-2 inhibitor celecoxib against hCAII, an isozyme with numerous physiological roles. The nanomolar CAI is linked to the pharmacophore SO2 NH2. We suggest that celecoxib may mediate some of its actions, e.g. anti-cancer effects, HCO3-modulation, by the COX-2 independent mechanism of CAI.

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THE PUTATIVE TUMOR SUPPRESSOR CDX2 IS OVER-EXPRESSED IN HUMAN COLONIC ADENOCARCINOMAS. M. Witek, J. Park, MD, PhD, R. Walters, K. Neilsen, S. Schulz, PhD, J. Palazzo, MD, S.A. Waldman, MD, PhD, Thomas Jefferson University, Philadelphia, PA.

Cdx2, an intestine-specific transcription factor, regulates genes that define the differentiated phenotype of enterocytes. Loss of Cdx2 has been suggested to underlie the progression of carcinomas in the colon and rectum. Here, expression of Cdx2 determined by quantitative reverse transcriptase-polymerase chain reaction (RNA) and immunohistochemistry (protein), in primary and metastatic colorectal tumors was compared to that in normal segments of the colonic epithelium. Surprisingly, Cdx2 was over-expressed by tumors, compared to normal mucosa in colon. Furthermore, Cdx2 was detected in lymph nodes containing metastatic cancer cells but not in those free of tumor. Thus, loss of Cdx2 expression does not contribute to mechanisms underlying neoplastic transformation of the colonic epithelium. Of clinical significance, Cdx2 is a molecular marker with utility for managing patients with colorectal malignancies.