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Identification of differentially expressed proteins in cigarette smoke condensate-induced lung cancer cells by comparative proteome analysis

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Lung cancer remains the leading cause of cancer death world wide. Exposures to many environmental agents including the inhalation of cigarette smoke, radioactive compounds, asbestos, heavy metals, and petrochemicals constitute the risk factors to lung carcinogenesis. Since cigarette smoking is one of the highest attributable risk factor for lung cancer, we attempted to identify potentially important proteins by employing the two dimensional gel electrophoresis (2DE)-based display proteomics. In this study, we report an approach to comparatively analyze differentially expressed protein profiles between the previously described cigarette smoking condensate (CSC)-induced tumorigenic human bronchial epithelial (HBE), 1170I and the pre-malignant 1799 and 1198 cell lines (Kim et al., Cancer Research 55: 5603, 1995). At least thirty proteins showed significant quantitative alterations between the tumorigenic and the pre-malignant cell lines. The altered proteins were characterized by matrix-assisted laser desorption/ionization (MALDI) coupled with time-of-flight (TOF) mass spectrometry (MS). Some of the identified proteins are known to regulate oxidative stress, cell cycle, or energy metabolism. Others are implicated in the lung cancer susceptibilities. We are currently investigating the potential significance of the some of these identified proteins in CSC-induced lung carcinogenesis. A functional and regulatory significance of Hsp90 up-regulation in 1170I cells is under extensive investigation since Hsp90 is known to function in the retinoic receptor signal transduction pathways and our previous study showed the retinoid refractoriness in tumorigenic 1170I cells. Identification of the repertoire of other altered proteins associated with CSC-induced lung carcinogenesis should provide additional insights into the mechanism of CSC-induced lung carcinogenesis as well as to provide useful biomarkers that can be employed as intermediate end points in the chemoprevention trials. [Supported by a grant from the Korean Ministry of Health and Welfare]

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Evaluation of the antineoplastic properties of COX-2 selective inhibitors in human breast and prostate tumor cell lines: evidence for a COX-2 independent mechanism

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Nonsteroidal anti-inflammatory drugs (NSAIDs) have striking cancer chemopreventive properties and may also have applications for advanced disease. For example, epidemiological studies have shown that NSAIDs can reduce the risk of death from colorectal cancer by as much as 40-50% and clinical trials are in progress to determine combination benefits with chemotherapy. The mechanism(s) for the antineoplastic properties of NSAIDs is controversial and may not involve cyclooxygenase (COX) inhibition. COX inhibition is, however, responsible for toxicity that limits the utility of NSAIDs for chemoprevention. Derivatives that are selective for the inducible COX-2 isozyme and have less GI toxicity than conventional NSAIDs are of particular interest for chemoprevention. In this study we determined the activity of the COX-2 selective inhibitors, celecoxib and rofecoxib, and the non-selective inhibitor, sulindac sulfide, in human MDA-MB-231 breast and LNCaP prostate tumor cell lines. Celecoxib and rofecoxib were synthesized at the IDD using published methods and structures confirmed by 1H- and 13C-NMR and mass spectrometry. Celecoxib inhibited the growth of MDA-MB-231 and LNCaP cells with IC_{50} values = 137 and 54 uM, respectively, as determined by the MTS assay. Celecoxib also induced apoptosis as measured by DNA fragmentation and displayed comparable potency with sulindac sulfide. By contrast, rofecoxib was essentially inactive in both cell lines at doses up to 500 uM. These results were unexpected because rofecoxib is a more potent than celecoxib with regard to COX inhibition and suggest a COX-independent mechanism of action. In addition, the greater sensitivity of prostate tumor cells to celecoxib compared to breast tumor cells indicates there may be important tissue-specific differences that are unrelated to the expression of COX-2 and should be tested in preclinical models before drugs are tested in clinical trials. Future studies are planned to evaluate these and other NSAIDs alone and in combination with standard chemotherapeutic drugs in xenograft and bone metastasis mouse models for advanced disease.

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NCX 4016, a nitric oxide-releasing aspirin derivative, exhibits a significant antiproliferative effect and alters cell cycle progression in human colon adenocarcinoma cell lines

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Purpose: Numerous studies demonstrated that non-steroidal anti-inflammatory drugs (NSAIDs) are useful agents for prevention and cure of cancers, especially colon and rectal cancers, but side effects are a major obstacle to their assuntion. Nitric oxide releasing non-steroidal anti-inflammatory drugs (NO-NSAIDs) are reported safer than NSAIDs by their ability to decrease gastric toxicity. In our study we assessed *in vitro* the cytotoxic activity of a new aspirin derivative, NCX 4016 [2-(Acetyloxy)benzoic acid 3-(nitrooxymethyl)phenyl ester], after different exposure schedules in three colon adenocarcinoma cell lines (LoVo, WiDr, LRWZ).
Experimental design: COX-1 and COX-2 expression of all the three cell lines was evaluated as protein expression and mRNA content by Western blot and RT-PCR respectively, cytotoxic activity was evaluated by sulforhodamine B assay and the data elaborated according to Monk's model, cell cycle perturbations and apoptosis were evaluated by flow cytometry, mitotic index was evaluated by at microscope on hematoxylin-eosin stained cytospin.
Results: All the lines were positive for the presence of protein and mRNA of the isoenzymatic form COX-1. Conversely, protein expression of COX-2 was limited to one (WiDr line). Important anti-proliferative effects were induced by NCX 4016 and GI50 value, ranging from 165-to 250 μM, was already reached after 24-h drug exposure in all cell lines. The antiproliferative action resulted independent from cell lineage, exposure time, and COX-1 or COX-2 status. A significant cell killing was observed only at the highest concentrations and LC50 values were reached only after longer time exposures. NO-aspirin compound also induced an accumulation of cells in G2-M phase in all the cell lines used with a peak after 48-h treatment which still persisted after 72-h or after 48-h exposure followed by a 24-h of wash-out. Furthermore, the block resulted be charged to G2 phase whereas mitosis phase was not affected at all.
Conclusion: Our results indicate that NCX 4016 has an *in vitro* antiproliferative activity superior respect to parental compound aspirin that makes it a potential important tumor chemopreventive agent and the cytocidal effect to higher concentration with specific block in G2 phase renders it a promising candidate for drug combination regimen.

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5-aza-2'-deoxycytidine and Trichostathin A enhanced growth inhibitory effect of all trans retinoic acid by the restoration of retinoic acid receptor β expression in head and neck squamous carcinoma cells

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The growth of head and neck squamous carcinoma cells (HNSCCs) are inhibited by all-trans retinoic acid (atRA) treatment. The effects of atRA on HNSCC growth and differentiation are mediated by nuclear retinoid receptors. However, the expression of retinoic acid receptor beta; (RAR-beta) is suppressed by aberrant methylation of RAR-beta promoter region in many HNSCCs, and acquire resistance to atRA treatment in such cells. These suppression of RAR-beta expression can be reactivated by exposure to demethylating agent, such as 5-aza-2'-deoxycytidine (5-AZA-CdR). Histone acetylation also plays a role in the control of expression of tumor suppressor genes. To investigate whether the reactivation of RAR-beta gene expression reverse retinoid resistance, atRA was co-treated with 5-AZA-CdR and histone deacetylase inhibitor (trichostatin A; TSA), either alone or in combination in RA-sensitive (1483) and RA-resistant (SqCC/Y1) HNSCCs. Cells were treated with 5-AZA-CdR at concentrations ranging from 0.05 microM to 5 microM 48 h.
After removing the media, we treated these cells with TSA 50 nM and 1 microM atRA sequentially for 48 h or 72 h. The cell viability was measured spectrophotometrically at 540 nm using the MTT assay. We measured cell cycle changes by the flow cytometric analysis. RAR-beta expression were analyzed using Western and Northern blotting. Methylation-sensitive PCR analysis was used to confirm that lack of expression of RAR-beta was due