# CIGARETTE SMOKE INDUCED TRANSFORMATION OF NORMAL BREAST EPITHELIAL CELL: ROLE OF ADENOMATOUS POLYPOSIS COLI IN LONG PATCH BASE EXCISION REPAIR

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Introduction: It was reported that environmental factor like smoking increases breast cancer. Chemical and animal bioassay studies indicate that tobacco smoke contains more than 4800 compounds and among 100 of these are proven mammary carcinogens. These carcinogens require metabolic activation to form intermediates that are often more reactive than the parent compound and can form DNA adducts. The DNA adducts are repair through two sub pathways (short patch (SP)BER and long patch (LP)BER) of Base Excision Repair (BER), that differ on the basis of repair gap size and the enzymes (APE, Pol β,γ,δ, Fen1, APC, DNA ligase and XRCC 1) involved in these pathways. XRCC1 has shown a positive association of long-term smoking with breast cancer risk. Adenomatous polyposis coli (APC) gene expression is increased in colon cancer by several DNA damaging agents and cigarette smoke carcinogen (DMBA), suggesting a possibility of interaction between APC and DNA repair machinery. It was also shown that APC interacts with pol-β, Fen1 and blocks LP-BER by blocking strand-displacement synthesis and then DNA repair.

The aim of the study is to check whether cigarette smoke condensate (CSC), a surrogate cigarette smoke is capable of transforming the normal breast epithelial cells, MCF10 A and delineate the biochemical basis for cell transformation and finally how APC plays a role for cell transformation by blocking DNA repair pathways.

Materials and methods: Maintenance of cells and CSC treatment:

The spontaneously immortalized MCF10A and other malignant and pre-malignant cancer cell lines were grown according to ATCC protocol. The CSC was prepared from the University of Kentucky Reference Cigarette 1R4F (9 mg tar and 0.8 mg. nicotine per cigarette). After cells reached sixty present confluence, they were treated with CSC as indicated time period

Growth assay, FACScan, and blot analysis: The anchorage dependent and independent growth of cells was measured by MTT and soft agar assay, respectively. DNA content of cells in different phases of the cell cycle was determined by propidium iodide staining methods by using flow Cytometer. The protein and RNA levels were determined by Western and Northern blot analysis, respectively.

DNA damage and repair assay (comet and luciferase assay): For evaluating DNA damage and repair of the cells after CSC treatment, a single-cell gel electrophoresis (comet assay) was performed as described by the manufacturer (Trivigen, Gaithersburg, MD, USA). For luciferase reporter assay the C residues of a plasmid DNA of p21(Waf-1/Cip1) promoter (pGL3-p21) was deaminated by 3M sodium bisulfite in the presence of 50 mM hydroquinone. The deamination of C produces U residues (U p21P, a substrate for SPBER). It was further treated with uracil DNA glycosylase and then reduced with 0.1M sodium borohydride to generate reduced AP-sites (Rp21P, a substrate for LPBER). Cells were transfected either p21P or Rp21P plasmid using FuGENE 6. The luciferase reporter activity was determined by using a Moonlight 3010 Illuminometer. The reporter activity was interpreted as the extent of DNA repair in these cells.

Overexpression and silencing of APC: The wild-type APC protein was overexpressed and silence by transient transfection of pCMV-APC and pSiRNAAPC plasmid, respectively.

Results: A single-dose treatment of normal breast epithelial cell line, MCF10A, for 72h with CSC resulted in a transformed phenotype. The anchorage-dependent growth of these cells was decreased due to increased cell cycle arrest in S to G2M phase; however, the surviving cells developed resistance due to an increased Bcl-xL to Bax ratio. Levels of PCNA, gadd45 and APC proteins (involved in DNA repair in response to genomic damage) were increased, suggesting that the cells were responding to CSC induced genomic damage. The transformation of MCF10A cells was determined by their colony forming efficiency in soft-agar in an anchorage independent manner. The reestablished cell lines from the soft agar when plated after several passages without CSC treatment, also formed colonies in the soft agar. With the use of a novel in vivo plasmid based LPBER assay it was shown that increased and decreased APC levels in different breast cancer cell lines were associated with a decrease or increase in LPBER activity, respectively. The effect of APC on LPBER in malignant and premalignant breast epithelial cell lines was produced by either overexpression or knockdown of APC. Furthermore, it was shown that the decreased LPBER in CSC-treated premalignant breast epithelial cells is associated with an increased level of APC and decreased cell growth.

Conclusion: The present study showed that the treatment with CSC transforms normal breast epithelial cell line. As CSC can generate abasic lesions in DNA that can be repaired by the BER pathway and a compromised BER can lead to accumulation of mutations and possibly transformation of normal breast epithelial cells. Thus it appears that CSC induced DNA damage increases APC protein level, which interacts with polβ to block polβ mediated strand-displacement synthesis of LP-BER and lead to cell transformation.