**Biological Basis for Radiation Adaptive Responses that Protect Against Bronchial Epithelial Cell Transformation**

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The major hypothesis in this project is that low-dose, low linear-energy-transfer (LET) radiation stimulates an adaptive response that protects cells from neoplastic transformation involving modulation of paracrine factors (e.g., cytokines), cell survival/death signaling pathways, and reprogramming of the epigenome. To test this hypothesis, a validated, sensitive *in vitro* transformation model and a media transfer method were used to study the mechanisms of low-LET gamma radiation activated natural protection (ANP) against chemical carcinogen-induced bronchial cell transformation. Immortalized human bronchial epithelial cell (HBEC) lines and BPDE, the active form of benzo(a)pyrene were used to mimic the DNA damage in the lungs of smokers. Macrophage and fibroblast cells, the main cell types found in microenvironment, were used to investigate the effect of LDR on production of tumor-promoting protein factors. A Gammacell-1000 Unit (Atomic Energy of Canada Ltd, Kanata, Ontario, Canada) was employed to irradiate the cells cultured in monolayers.

**A. Low Dose Gamma-rays protect against BPDE-induced cytotoxicity in bronchial epithelial cells**

Low dose radiation (LDR; 30, 60 or 90 mGy) alone exerted marginal effects on proliferation and survival in the immortalized (HBEC2 and Beas-2B) and precancerous (HBEC-2B, transformed by treatment with BPDE based on colony formation in soft agar, but unable to form tumors in nude mice) cell lines. However, LDR had a protective effect against BPDE-induced cytotoxicity in Beas-2B cells, consistent with previous reports that LDR protects cells from death induced by DNA damage from high-dose radiation. As for precancerous cells, in contrast to the untransformed cells, LDR had no obvious protection against BPDE-induced cytotoxicity. These results suggest that the effects of LDR on normal and transformed bronchial epithelial cells are different. Further studies will now address the key question as to whether chronic (weekly) LDR can modulate transformation efficiency in HBECs. In addition, recent work from our group has shown that as short as 4-weeks exposure to the carcinogens induces a multifaceted dedifferentiation involving epigenetically regulated epithelial to mensenchymal transition (EMT). Thus, we will have several endpoints to assess the mechanisms of LDR's protective effect against BPDE-induced cell transformation in HBEC cells.

**B. Modulation of tobacco carcinogen-induced microenvironmental cytokines, chemokines, and growth factors that facilitate lung carcinogenesis by LDR**

We hypothesized that genotoxic tobacco carcinogens induce lung tumor development through genetic and epigenetic changes within epithelial cells and through promoting a microenvironment that favors survival and proliferation of precancerous cells. Influencing factors can be secreted from bronchial epithelial cells, inflammatory cells such as macrophages and stromal cells such as fibroblasts. LDR may suppress carcinogenesis through modulating the chemical-carcinogen-altered tumor-promoting microenvironment. Antibody arrays interrogating 40 growth factors and 39 cytokines and chemokines were used to detect the factors secreted in the culture media from HBEC-2

(epithelial cells), THP-1 (macrophages) and HFL-1 (lung fibroblasts). ELISA was used to validate results.

The results show that BPDE stimulates release of numerous cytokines and growth factors from each cell type. In THP-1 cells, secretion of IL-1β, IL-1α, TNF-α, IL-6, GM-CSF, CXCL-5, MCP-2, GCP-2 and FGF-6 was induced while in HBEC-2 cells, production of HGF, TGF-α and TGF-β was observed. In contrast, in HFL-1 cells, secretion of CXCL5, CXCL1, IL-6, VEGF, FGF-4, and bFGF was induced by BPDE. These factors are reported to be involved in promoting carcinogenesis and thus, could contribute to lung cancer development.

The effect of LDR on BPDE-induced secretion of cancer promoting factors was then examined. Significant inhibition of BPDE-induced IL-6, CXCL1 and CXCL5 secretion from HFL-1 cells was observed, while LDR had marginal effects on secretion of protein factors from THP-1 and HBEC-2 cells. Although IL-6 is recognized as an important cytokine contributing to cancer progression, its role in promoting cell transformation is unknown. Therefore, we then focused on the effect of LDR in modulating IL-6 secretion during BPDE-induced cell transformation.

**C. The role of modulating BPDE-induced IL-6 secretion from fibroblasts by LDR on cell transformation**

Carcinogens derived from cigarette smoke such as BPDE and NNK induce lung cancer through mutations and epigenetic silencing of tumor suppressing microRNAs and other protective genes. However DNA damage can also be beneficial in that it also activates DNA repair pathways and apoptosis that eliminate genomically unstable cells. Therefore, cancer development and progression likely depend on the balance between aberrant precancer (transformed) cell survival and apoptosis signals. Thus, we hypothesize that IL-6 protects the DNA mutated cells and cells with epigenetically silenced protective genes from death and promotes cell transformation.

We first confirmed the BPDE-induced IL-6 secretion from HFL-1 cells by ELISA. A BPDE-dose-dependent induction was effectively suppressed by LDR (30, 60 or 90 mGy). With chemical inhibitors, we determined that NF-κB and ERK are the main pathways mediating this induction. A medium transfer assay was conducted, which showed that the conditioned media (CM) from the BPDE-treated HFL-1 cells protected the precancerous HBEC-2B cells against BPDE-induced cytotoxicity. Further experiments with recombinant IL-6 confirmed that this cytokine protects against BPDE-induced cytotoxicity in HBEC-2B cells. In addition, IL-6 robustly activated several cell survival signals such as STAT3, ERK and JNK in HBEC-2B cells. These results confirm that IL-6 promotes survival of precancerous cells, supporting our hypothesis that LDR alters the tumor-promoting microenvironment to repress lung carcinogenesis, and that reduced IL-6 secretion from pulmonary fibroblasts is involved.

Ongoing experiments are designed to determine if IL-6 from the CM potentiates BPDE-induced cell transformation and the mechanism by which LDR suppresses BPDE-induced IL-6 secretion from pulmonary fibroblasts. In addition, the effect of chronic LDR on these cell signaling pathways, induction of EMT and transformation in response to tobacco carcinogens will be determined. Taq-SNPs capturing sequence variation in these cytokines and growth factors will assess their association to risk for squamous cell carcinoma in former uranium miners under Project 3 of this Program Project.

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