Microarray Analysis of Curcumin in HNSCC
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Abstract

Overall survival for HNSCC has not significantly improved. Treatment failures predominantly develop due to local-regional recurrences and second primaries as a result of field-carcinoma from tobacco use. Identifying a chemopreventive agent that ablates this process could affect survival outcomes. Curcumin, a dietary minor extract, is an antioxidant in cancer clinical trials in a complex form as Curcumin C3 (C), although the effect on in vivo HNSCC has not been investigated. Understanding the cellular response to curcumin may identify a novel mechanism-based chemopreventive. Several oral and hematologic cancers, although its effect on oral HNSCC has been limited for clinical trials in a complex form as Curcumin C3 ®, although its effect on regional recurrences and second primaries as a result of field cancerization from tobacco use. Identifying a novel mechanism-based chemopreventive strategy for malignancies with a wide range of molecular abnormalities.

Methods and Materials

A product isolated from turmeric, curcumin has been implicated as a powerful therapeutic in a variety of human cancers (1) (because of its ability to induce apoptosis and is currently undergoing clinical trials for colon, skin, pancreatic, and hematologic cancers, although its effect on oral HNSCC has not been investigated for in vivo HNSCC). Laboratory data indicate that curcumin can inhibit tumor initiation, promotion, invasion, and metastasis (2). Curcumin inhibits cell proliferation by interfering with the mTOR pathway that mediates proliferation and cell survival, and has been shown to induce apoptosis of tumor cells by inhibiting the mTOR pathway (3). The mTOR pathway is a key signaling pathway that regulates cap-dependent translation as well as apoptosis through phosphorylation of S6K and subsequent death signaling of Bad, which allows association of Bcl-2/Bcl-xL to initiate activation of the caspase pathway and cleavage of PARP.

Cell line and origin: FaDu, SCC16, SCC25, SCC066, SCC116; HNSCC cell lines kindly provided by Dr. Susan Gollin (University of Pittsburgh), and SCC114-FOM, PCI-13, PCI-15a, PCI-30; HNSCC cell lines kindly provided by Dr. Teresa Whiteside (University of Pittsburgh). HNSCC cell lines were grown in the presence of curcumin or C3 (0-40 μM) and analyzed with GeneSpring 6.1. 100 μM curcumin or C3 was selected for our studies. Curcumin has currently being evaluated in a phase I trial of metastatic colon cancer, and was selected for our studies. Curcumin has been shown to effect various cell cycle proteins and checkpoints downregulation of some of the cyclins and cyclin-dependent kinases, upregulation of cell death inhibitors, and inhibition of DNA synthesis (reviewed in 6) in a variety of human cancers. Curcumin has previously been shown to induce G2/M phase cell cycle arrest in the MDA-MB-231 breast cell line (7). Therefore we explored the role of caspase plays in the cell cycle from a variety of HNSCC cell lines. Curcumin is expected to affect events through the mTOR pathway, i.e. regulation of cell growth and survival, in prostate, glioma, and breast cancer. Curcumin has also been shown to induce apoptosis of tumor cells in HNSCC, although the modulation of mTOR, a transcription factor that controls cell proliferation and cell survival, by curcumin in HNSCC has not been studied.

Results

Figure 1. Growth inhibition of HNSCC cells in vitro with curcumin. Curcumin C3 inhibits proliferation of tumor cells FaDu, PCI15a, PCI13, SCC16, SCC25, SCC066, SCC114-FOM, MIKR in a dose-dependent manner. Note that cells are consistently sensitive to killing by curcumin at 0-10μM, a physiologically relevant concentration.

Figure 2. Flowcensis activated sorting (FACS) analysis of HNSCC cells treated with curcumin. FACS analysis was done on HNSCC cell lines treated with 10μM C, a concentration inducing cell death in all cell lines analyzed by MTT assay. A, peak at 0.20 corresponds to DNA in the G1 phase of the cell cycle, and a peak at 0.40 corresponds to the G2/M phase. A peak at 0.55 corresponds to a G1/S cell cycle arrest, whereas other cell lines did not consistently demonstrate any cell cycle arrest with curcumin treatment.

Conclusions

A total of 143 curcumin were found to be differentially expressed after 24 hours of curcumin exposure, of which 102 genes were upregulated and 41 were downregulated. A total of 147 genes were found to be differentially expressed after 48 hours of curcumin exposure, of which 127 genes were upregulated and 40 genes were downregulated. Some of the most notably differentially expressed genes were involved in the cell cycle (P < 2), NFκB (and therefore apoptosis and survival), phosphorylation of ERK1/2, oxidative state of the cell, and protein couples receptors (GPCR), cell morphology/shape, extracellular matrix, and osteoclasts. These results indicate that curcumin affects many components of the mTOR and NFκB signaling pathways but does not affect mTOR directly at the level of mTOR. Curcumin inhibits the mTOR pathway that mediates proliferation and cell survival, and has become a target foranticancer therapies through the use of rapamycin and its derivatives (3). Targeting mTOR pathway with rapamycin and rapamycin analogues such as CCI-779, RAD-001, and ATP2073 inhibits cell cycle progression, cell growth, and proliferation signalling. In cancer cells, these pathways may be constitutively activated. Clinical trials have demonstrated tumor growth suppression against a variety of human cancers with rapamycin and its analogues. Therefore, development of inhibitors of mTOR and related pathways is a rational chemopreventive strategy for malignancies with a wide range of molecular abnormalities.

Bibliography

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