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ORIGINAL RESEARCH

Reduction of p53 by Knockdown of the *UGT1* Locus in Colon Epithelial Cells Causes an Increase in Tumorigenesis

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SUMMARY

UGT1A expression is required to maintain and sustain p53 activation in stress-induced colon epithelial cells, and it has a significant impact on p53-mediated apoptosis and tumor suppression.

BACKGROUND & AIMS: The UDP-glucuronosyltransferases (UGTs) are a part of the cell machinery that protects the tissues from a toxicant insult by environmental and host cell metabolites. We investigated the mechanism behind tumor growth and UGT repression.

METHODS: We initially silenced the *Ugt1* locus in human colon cell lines and investigated markers and responses linked to p53 activation. To examine the role of the *Ugt1* locus in p53-directed apoptosis and tumorigenesis, experiments were conducted to induce acute colon inflammation and chemically induced colon cancer in mice where we have selectively deleted the *Ugt1* locus in the intestinal epithelial cells ($Ugt1^{\Delta IEC}$ mice).

RESULTS: Knockdown of the UGT1A proteins by RNAi in human colon cancer cells and knockout of the *Ugt1* locus in intestinal crypt stem cells reduces phosphorylated p53 activation and compromises the ability of p53 to control apoptosis. Targeted deletion of intestinal *Ugt1* expression in $Ugt1^{\Delta IEC}$ mice represses colon inflammation-induced p53 production and proapoptotic protein activation. When we induced colon cancer, the size and number of the tumors were significantly greater in the $Ugt1^{\Delta IEC}$ mice when compared with wild-type mice. Furthermore, analysis of endoplasmic reticulum (ER) stress-related markers indicated that lack of UGT1A expression causes higher ER stress in intestinal epithelial cells and tissue, which may account for the lower expression of p53.

CONCLUSIONS: Our results demonstrate that *UGT1A* expression is required to maintain and sustain p53 activation in stress-induced colon epithelial cells and has a significant impact on p53-mediated apoptosis and tumor suppression, thus protecting the colon tissue from neoplastic transformation. *(Cell Mol Gastroenterol Hepatol 2016;2:63–76; http://dx.doi.org/10.1016/j.jcmgh.2015.08.008)*

Keywords: Apoptosis; Colon Cancer; ER Stress; UGT1A.

Colorectal cancer (CRC) ranks as the third most common cancer worldwide and the second leading cause of cancer-related deaths in Western society.[1,2] The majority of colorectal tumors are epithelial tumors, whereas lymphomas, endocrine tumors, and mesenchymal tumors are quite uncommon.[3] As an important extrahepatic tissue of xenobiotic metabolism, the colorectum is in direct contact with xenobiotic substances, including potentially toxic or carcinogenic agents, presumably leading to the high incidence rate of CRC.[4,5] By contrast, cancers of the small intestine are rarely seen, even though the small intestine has a larger mucosal surface area than the colorectum.[6] One plausible explanation is that expression of biotransformation enzymes, including glutathione S-transferases, UDP-glucuronosyltransferases (UGT), and cytochrome P450, are lower in the colorectum than in the small intestine. These enzymes are responsible for the detoxification of ingested toxins, carcinogens, or tumor-promoting compounds, and their lower expression levels in colorectum are considered to be a contributing factor to the high rate of CRC.[7,8]

As an important part of the detoxification process, glucuronidation provides an effective metabolic process leading toward the biological inactivation of potential toxicants and carcinogens. Previous studies have demonstrated that gastrointestinal UGT activity decreases sharply from the small intestines to the colon tissue.[7] This decrease in UGT activity contributes to higher colonic DNA damage caused by carcinogens, such as heterocyclic amines and polycyclic aromatic hydrocarbons, which are usually detoxified through UGT glucuronidation.[9–11] It has been speculated that glucuronidation provides a genoprotective defense

Abbreviations used in this paper: **AOM, azoxymethane; CRC, colorectal cancer; DSS, dextran sodium sulfate; ER, endoplasmic reticulum; IEC, intestinal epithelial cells; IL, interleukin; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBS, phosphate-buffered saline; qRT-PCR, quantitative real-time polymerase chain reaction; siRNA, small interfering RNA; TNF, tumor necrosis factor; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; UGT, UDP-glucuronosyltransferase; *UGT1*, human *UGT1* locus; *Ugt1*, murine *Ugt1* locus.**

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against the mutagenic actions of chemical carcinogens. Studies have found that UGT expression in colorectal tumor tissues is significantly reduced in comparison to surrounding healthy tissues.[12–14] Indeed, the pattern of UGT down-regulation is also identified in other types of cancer, including liver and biliary cancer,[15] breast cancer,[16] and bladder cancer.[17] These findings indicate that UGT expression is reversely correlated with tissue neoplastic transformation. However, there is no evidence that the UGTs impact the outcome of tumorigenesis, and the underlying mechanism regarding the role of the UGTs in cancer development is largely unexplored.

Recent studies have shown a link between the UGTs and p53, an important regulator of cell cycle, apoptosis, and tumorigenesis. Ariyoshi et al[18] observed increased constitutive UGT1A activity in $p53^{+/-}$ mice, and Hu et al[19] verified that epirubicin up-regulates UGT2B7 expression via a p53 pathway. In contrast to these studies, our initial discovery uncovered an opposite causal relationship between UGT1A and p53 when we challenged cells with chemical stress. Cell apoptotic death is a well-defined mechanism that is associated with cancer suppression when the body encounters tumor-promoting challenges. P53 and its signaling network are known to play a critical role in the regulation of the cell cycle and apoptosis to conserve gene stability, thus suppressing tumor development.[20,21] Upon occurrence of cellular stress, such as oncogene activation or DNA damage, p53 is activated. When cell damage is minimal, p53 evokes cell cycle arrest by inducing p21 to promote DNA repair and cell survival, whereas sustained p53 activation in response to high damage levels triggers cellular apoptosis, thus preventing the expansion of damaged cells and protecting normal tissue from neoplastic transformation.[22,23] This is evidenced by the fact that the development of certain tumors in p53 null mice has been associated with decreased apoptosis, implying the important role of p53 in promoting cell death during tumor suppression.[24]

Our study explored the role of the UGT1A proteins in CRC by using colon cancer cell lines and an intestinal conditional knockout animal model deficient in *Ugt1* locus expression. By documenting molecular and cellular events that are associated with p53-dependent signaling, this study sheds light on the importance of UGT1A expression on p53-dependent stress responses and tumor suppression.

Materials and Methods

Chemicals and reagents

Actinomycin D, etoposide, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and azoxymethane (AOM) were purchased from Sigma-Aldrich (St. Louis, MO), and dextran sulfate sodium (DSS, molecular weight 36,000–50,000) was obtained from MP Biomedicals (Santa Ana, CA). The quantitative real-time polymerase chain reaction (qRT-PCR) primers were commercially synthesized from Integrated DNA Technologies (San Diego, CA). Antibodies against UGT1A (Abcam, Cambridge, MA), p21 (Chemicon, Temecula, CA), p53 and Bax (Santa Cruz Biotechnology, Dallas, TX), and caspase-9 and caspase-3 (Cell Signaling Technology, Beverly, MA) were used in Western blot analyses.

Cell Culture and UGT1A Silencing

The human colon epithelial cell lines HT29 and LS180 were obtained from the American Type Culture Collection (ATCC, Manassas, VA). For *UGT1A* gene silencing, two pairs of *UGT1A*-specific small interfering RNA (siRNA) designed along with nonspecific siRNA as a negative control were used. The siRNA (Invitrogen/Life Technologies, Carlsbad, CA) was mixed with the Lipofectamine RNAiMAX Reagent (Invitrogen) in Opti-MEM I medium (GIBCO/Life Technologies, Grand Island, NY) at a finial concentration of 30 nM and was incubated at room temperature for 10 minutes according to the manufacturer's instructions. The mixture was added to culture plates, and exponentially growing cells were then seeded in these plates. Cells were incubated for 24–72 hours until further analyses.

Mouse Crypt Cell Preparations and Culture

Crypt cell isolation and culture were performed as previously described elsewhere.[25] Intestines from adult $Ugt1^{F/F}$ and $Ugt1^{\Delta IEC}$ mice were dissected, opened longitudinally, and gently washed with ice-cold phosphate-buffered saline (PBS) buffer. Intestinal tissue was then incubated in PBS buffer containing 2 mM EDTA at 4°C for 30 minutes. The buffer was removed, and the tissue was shaken vigorously and then filtered through a 70-μm cell strainer. The filtrate was centrifuged at 1000*g* for 10 minutes to precipitate the crypt cells, followed by a wash with Advanced Dulbecco's modified Eagle medium/Ham's F-12 medium (Life Technologies, Carlsbad, CA). The cells were counted, and approximately 1000 crypts were suspended into 50 μL of Matrigel (BD Biosciences, San Jose, CA), and the cells plated into 24-well plates. After 10 minutes, 500 μL of crypt culture medium Advanced Dulbecco's modified Eagle medium/Ham's F-12 supplemented with B27 (Life Technologies), N2 (Life Technologies), 1 μM *N*-acetyl cysteine (Sigma-Aldrich), 100 ng/mL mNoggin and R-spondin 1 (conditioned medium, R-spondin 1 expression 293-HA-Rspol-Fc cell line was a generous gift from Dr. Calvin Kuo, University of Stanford) were added. Growth factors were added every other day, and the medium was changed every 4 days. Cells were passaged every 1 to 2 weeks.

Cytotoxicity Assay

Cells were seeded at 7000 cells per well to a 96-well plate and incubated overnight. The cells were subsequently exposed to the indicated concentrations of actinomycin D or etoposide. After 36 hours, the MTT solution was added to each well at a final concentration of 0.5 mg/mL, and the plate was incubated at 37°C for another 4 hours. The MTT solution was then removed and 150 μL of DMSO per well was added. The absorbance at 595 nm was measured by a microplate reader.