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Relationships between genomic, cell cycle, and mutagenic responses of TK6 cells exposed to DNA damaging chemicals

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Abstract

Genotoxic stress causes a variety of cellular and molecular responses in mammalian cells, including cell cycle arrest, DNA repair, and apoptosis. These responses result from the interplay between the genotoxic events themselves, and the biological context in which they occur. To better understand this interplay, we investigated cytotoxicty, mutagenesis, cell cycle profile, and global gene expression in the human TK6 lymphoblastoid cell line exposed to six genotoxicants. The six compounds have broad structural diversity and cause genotoxic stress by many different mechanisms, including covalent modification (methyl methanesulfonate, mitomycin C), reactive oxygen species (hydrogen peroxide, bleomycin), and topoisomerase II inhibition (etoposide and doxorubicin). Cell cycle analysis was performed 4 and 20 h following a 4 h chemical exposure. Cells exposed to all compounds experienced S-phase arrest at the 8 h time point, but by 24 h had markedly different cell cycle responses. Cells exposed to compounds that cause covalent modification had a strong G_2/M arrest at 24 h. These cells also had a robust (>25-fold) increase in mutant frequency, and had a moderate but sustained p53 response at 4, 8, and 24 h, detectable as ~2–5-fold increases in transcript levels for $p21^{WAF1/CIP1}$, GADD45α, BTG2, and cyclin G1. In contrast, cells exposed to the reactive oxygen compounds had little or no G_2/M arrest at 24 h and no increase in mutant frequency. In addition, these compounds caused a strong but transient induction of the p53 pathway, detectable as 15–25-fold increases in $p21^{WAF1/CIP1}$ transcription at 4 h that decreased dramatically by 8 h and was near control levels at 24 h. Thus, the mutagenic effect of compounds was consistent with G_2/M arrest and sustained kinetics of p53 pathway activation. Global gene expression data were also consistent with the mutagenesis data. Activation of genes associated with cell cycle arrest, the p53 and TNF-related pathways, and chemokines and chemokine receptors, were particularly

Abbreviations: MMS, methylmethanesulfonate; MMC, mitomycin C; ETOP, etoposide; DOX, doxorubicin; H_2O_2, hydrogen peroxide; BLEO, bleomycin sulfate; QPCR, quantitative real-time PCR; MF, mutant frequency; MI, mutant index; RSG, relative suspension growth; TK, thymidine kinase; TNF, tumor necrosis factor

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evident for the reactive oxygen compounds. In contrast, the most mutagenic compounds caused fewer and less robust changes in global gene expression. There was therefore an inverse relationship between global gene expression and mutagenic potency.

Keywords: TK6 cells; Microarray; Cell cycle; Genotoxicity; Toxicogenomics

1. Introduction

Genotoxic compounds cause DNA damage by a variety of mechanisms. Direct-acting chemicals that bind covalently to DNA include DNA alkylating agents, cross-linking agents, and oxygen radicals, such as MMS, MMC, and H_2O_2, respectively [1,2]. Indirect-acting genotoxicants, on the other hand, alter the function of cellular proteins, leading to accumulation of endogenous DNA damage. An example of this is the accumulation of DNA strand breaks that results from inhibition of topoisomerase activity by the anti-neoplastic agents ETOP and DOX. Unless repaired by the cell, each type of DNA damage caused by these agents can provoke mutations.

Mammalian cells repair DNA damage by many biochemical mechanisms, including base excision repair, nucleotide excision repair, and recombination repair [3]. Cooperation and functional redundancy between these pathways is known, but in general the different repair mechanisms specialize in repairing specific classes of DNA damage. Bases modified by methylation and oxidation are removed by glycosylases, producing abasic sites that are eliminated through the base excision repair pathway. Larger base modifications (e.g., bulky adducts) are removed via nucleotide excision repair. DNA strand breaks are repaired by recombination pathways, such as non-homologous end joining and, less commonly in mammalian cells, homologous recombination. Each of these processes is facilitated by additional cellular responses to genotoxic stress, including those that detect DNA damage and lead to cell cycle arrest [3].

Genotoxic stress triggers a variety of cellular responses including the transcriptional activation of genes regulating DNA repair, cell cycle arrest, and apoptosis [4–7]. The cellular responses to genotoxic stress caused by radiation and chemical-induced DNA damage is partly mediated by the activation of signal transduction pathways involving mitogen-activated protein kinases (MAPKs), including the extracellular signal-regulated kinases (ERKs), the c-Jun NH2-terminal kinases (JNKs), and the p38 kinases. All of these pathways influence the activities of transcription factors, among which the best understood are tumor suppressor p53 and NF-κB [8–12]. p53 is widely considered to be the major sensor of genotoxic stress and is a critical link between DNA damage, cell cycle arrest, and apoptosis. Cells with wild-type p53 typically respond to genotoxic stress by arresting the cell cycle and repairing damaged DNA, followed by survival or apoptosis. Cells lacking normal p53 function are known to experience greater genomic instability as a consequence of a reduced ability to undergo cell cycle arrest and apoptosis. These phenotypes help explain why p53 is the most commonly mutated gene in human cancer [9,13–15].

NF-κB activation has been implicated as a key cellular response that modulates the outcome of cells exposed to radiation and genotoxic agents [16–18]. It is known that NF-κB regulates the transcription of genes critical for a variety of biological processes, including immune response, inflammatory reactions, and apoptosis [19,20]. NF-κB has been implicated in both negative and positive regulation of cell death, can be activated by treatment with tumor necrosis factor (TNF) and various chemical agents, and may affect mutagenesis [16–20]. However, only a small number of experiments have been reported in which both mutagenesis and global gene expression analysis have been examined.

DNA microarrays give researchers the ability to measure the expression levels of thousands of mRNA transcripts simultaneously [21,22]. Among the many applications of microarrays has been advancement of our understanding of how organisms respond to toxic injury [23–26]. For example, investigators have reported profound effects of genotoxic and environmental stressors on global transcriptional responses in yeast. MMS caused altered gene expression for up to 14% of the genes in the yeast genome, including genes responsible for detoxification, mitochondrial functions, metabolite transport, and biosynthesis