Singlet Molecular Oxygen Generated In Situ Into DNA Induces Proteome Alterations in Immortalized Keratinocytes

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Thiopurines, such as 6-thioguanine (6-TG), are employed in immunosuppression and autoimmune disease therapy. Metabolism through the purine salvage pathway yields thioguanine nucleotides, which can incorporate into DNA, disrupting cell replication by exerting a cytostatic effect. It is important to note that thiopurine treatment may pose an elevated risk of skin cancer due to 6-TG photosensitization by UVA radiation present in sunlight (320 - 400 nm). This process triggers the formation of singlet molecular oxygen by 6-TG with a relatively high quantum yield (approximately 0.6), which has been associated with potential skin cancer risk in patients. Recent studies have demonstrated that a low dose of UVA (6 J/cm²) can cause cell proteome remodeling and induce senescence in keratinocytes. Accordingly, this work aims to understand the consequences of singlet oxygen generation through 6-TG photosensitization *in situ* within DNA by exploring proteome alterations induced by this oxidant. Human immortalized keratinocytes (HaCaT cells) were treated with 6-TG, followed by UVA exposure. Three control experiments were performed simultaneously: 1) untreated cells kept in the dark; 2) cells treated with 6-TG and kept in the dark; 3) untreated cells exposed to 6 J/cm² of UVA radiation. After cells were lysed, their proteins were extracted, digested into peptides and analyzed by LC-MS/MS. Hierarchical clustering analysis demonstrated a distinct separation among the four experimental conditions, each characterized by varying protein abundances. Further enrichment analyses revealed that UVA-irradiated cells displayed terms associated with protein degradation and chromatin regulation. In contrast, cells that had 6-TG incorporation, and underwent UVA irradiation exhibited enriched terms linked to a broad network of proteins involved in DNA damage and redox stress response. Our results pointed to alterations in the subcellular proteome, in particular in the nucleus and in the mitochondria, in cells treated with 6-TG and further subject to irradiation. They also suggest that the formation of DNA damage induced by 6-TG photosensitization can contribute to cellular dysfunction, and potentially to the high risk of cancer in patients treated with this drug.

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