Synopsis of Original Research Paper

A new method for detecting new biological risk assessment of chemical substance using live cell analysis

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Risk assessment studies of chemical substances that induce DNA lesions are important, because DNA lesions in genomic DNA result in cancer in humans. Many classes of DNA lesions induced by chemical agents are eliminated via DNA repair nucleotide excision repair (NER) for the maintenance of genomic integrity. Ames test is one of the most commonly applied tests in toxicology. In the test, NER-deficient mutant bacterial cells are used to detect the mutagenic capacity of chemical substances. Unscheduled DNA synthesis test is also popular test for toxicology and measures DNA repair synthesis after excision of oligonucleotides containing DNA lesions during NER. And in human, individuals with NER-defective xeroderma pigmentosum are cancer-prone. For toxicology research in human, therefore, it might be important to identify NER-repairable bulky DNA lesions induced by chemical substances. So far, simple and quick assays to detect such damaging agents have not been developed using human living cells. Here, we report a simple, non-isotopic assay for determining DNA damaging agents that induce NER-repairable DNA lesions by visualizing gene expression from treated fluorescent protein vectors in a mammalian living cell system. This assay is based on a comparison of fluorescent protein expression in NER-proficient and NER-deficient living cells. When we tested UV-irradiated fluorescent protein vectors, the fluorescent protein was observed in NER-proficient living cells, but not in NER-deficient living cells. Similar results were obtained for vectors treated with the anticancer drug, cisplain. By contrast, when treated with the DNA alkylating agent methyl methanesulfonate, believed to cause no NER-repairable lesion, no difference in gene expression was observed in between NER-proficient and NER-deficient living cells. Using fluorescent protein expression and living test cells, our assay can specifically detect DNA-damaging agents that induce NER-repairable DNA lesions, and could be used to analyze chemicals with the potential to cause cancer.