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An Improved Method for Detecting DNA Repair by Using Alkaline Comet Assay

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The alkaline comet assay mainly detects DNA single-strand breaks (SSBs) in cells. However, as rejoining of DNA SSBs by most cell types is known to be a rapid process with a halftime of a few minutes, it is usually difficult to follow up the DNA repair speed with a traditional way in alkaline comet assay.

In the present study, we tried a new method of treatment of cells and observed DNA repair level in 4,000-6,000 human fresh blood leukocytes at 0, 1, 2, 5, 10 and 30 minutes on the basis of alkaline comet assay. The result showed that, the untreated blood leukocytes (baseline) had an average of 1.9% of DNA in the "comet" tail, and the treated ones (bleomycin, 0.5μg/mL×5 min, 4℃) had an average of 59.7% of DNA in the "comet" tail. After 1, 2, 5, 10, and 30 minutes of culture of the treated leukocytes in bleomycin-free RPMI 1640 (37℃), the amount of DNA in the "comet" tail decreased to 49.4%, 44.5%, 13.3%, 11.5% and 1.3%, respectively, with a halftime of decrement (repair) $t_{1/2}$ = 3.38 min. We estimated cell numbers and total DNA amount in the comet image and would explain that the decrement of DNA in "comet" tail should be resulted from DNA repair.

In Summary, the improved method (1) used a lowest concentration of bleomycin and induced a highest percentage of DNA in comet tail among those previously reported; (2) used the shortest treatment time of cells and shortest time interval in observing DNA repair among those reported; (3) handled multiple samples simultaneously; (4) strictly controlled the variation of treatment time and repair time within seconds.

In conclusion, we suggest this method find a wider application in future studies.

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Development of a Simple Mutagenicity Test Using eGFP-MDC1-expressing Cells

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Phosphorylation of histone H2AX at S139 (γ-H2AX) is used as a marker for DNA damage in cell level. γ-H2AX is typically detected by Western blotting or immunostaining in mutagenicity test. However, these methods require tangled handling, investment of time, and an expensive anti-γ-H2AX antibody. MDC1 (Mediator of DNA damage checkpoint protein 1) is a nuclear adaptor protein which binds to γ-H2AX and recruit several DNA damage response proteins to DNA damage site. Thus MDC1 forms foci in nuclei in response to DNA damage as well as γ-H2AX.

In this study, we propose a simple mutagenicity test using eGFP (Enhanced green fluorescent protein)-MDC1-expressing cells. The experimental procedures are: ① exposure of test chemicals, ② photographing of eGFP (MDC1) fluorescence, and ③ measurement of the frequency of foci formation. Discrimination and count of foci-forming and normal cells is automated by R, which is a free software for statistics analysis, and machine learning. Our method may be applicable to screening test for chemical mutagenicity.

eGFP-MDC1 発現細胞を用いた簡便な変異原性試験法の開発

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ヒストン H2AX の S139 のリン酸化(γ-H2AX)は細胞レベルの DNA 損傷の指標として化学物質の変異原性試験に汎用されている。しかしその検出には煩雑で時間がかかるウエスタンブロッティングや免疫染色が用いられており、その際に使用する抗γ-H2AX 抗体も高価である。MDC1 (Mediator of DNA damage checkpoint protein 1)はγ-H2AX に結合して種々の DNA 損傷応答タンパク質を DNA 損傷部位に呼び寄せる核タンパク質で、γ-H2AX と同様に DNA 損傷に応じて核内で Foci を形成する。

本研究では eGFP (Enhanced green fluorescent protein)-MDC1 発現細胞を用いた簡便な変異原性試験法を提案する。試験の流れは、①被験物質の曝露、②eGFP (MDC1)の蛍光の撮影、③MDC1 の Foci 形成頻度の計測である。③では統計解析用の無料ソフトウェア R を用いて、機械学習による Foci 形成細胞の判別、計測の自動化を図った。この方法は蛍光顕微鏡以外に高価な装置、試薬が不要の為に、化学物質の変異原性のスクリーニング試験に適用できると考えている。