Development and Application of a Slot-Blot Assay Using the Damage Sensing Protein Atl1 to Detect and Quantify O^6-Alkylated Guanine Bases in DNA

By Yaakub, H (Yaakub, Hanum) [1] ; Howell, A (Howell, Anthony) [2] , [3] , [4] ; Margison, GP (Margison, Geoffrey P.) [1] ; Povey, AC (Povey, Andrew C.) [1]

View Web of Science ResearcherID and ORCID (provided by Clarivate)

Source TOXICS

Volume: 12 Issue: 9

DOI: 10.3390/toxics12090649

Article Number 649

Published SEP 2024

Indexed 2024-10-10

Document Type Article

Jump to ↓ Enriched Cited References

Abstract Humans are unavoidably exposed to numerous different mutagenic DNA alkylating agents (AAs), but their role in the initiation of cancers is uncertain, in part due to difficulties in assessing human exposure. To address this, we have developed a screening method that measures promutagenic O-6-alkylguanines (O-6-AlkGs) in DNA and applied it to human DNA samples. The method exploits the ability of the Schizosaccharomyces pombe alkyltransferase-like protein (Atl1) to recognise and bind to a wide range of O-6-AlkGs in DNA. We established an Atl1-based slot-blot (ASB) assay and validated it using calf thymus DNA alkylated in vitro with a range of alkylating agents and both calf thymus and human placental DNA methylated in vitro with temozolomide (TMZ). ASB signals were directly proportional to the levels of O-6-meG in these controls. Pre-treatment of DNA with the DNA repair protein O-6-methylguanine-DNA methyltransferase (MGMT) reduced binding of Atl1, confirming its specificity. In addition, MCF 10A cells were treated with 500 mu M TMZ and the extracted DNA, analysed using the ASB, was found to contain 1.34 fmoles O-6 -meG/mu g DNA. Of six human breast tumour DNA samples assessed, five had detectable O-6-AlkG levels (mean +/- SD 1.24 +/- 0.25 O-6-meG equivalents/mu g DNA. This study shows the potential usefulness of the ASB assay to detect and quantify total O-6-AlkGs in human DNA samples.

Keywords **Author Keywords:** N-nitroso compounds; O-6-alkylguanines; Atl1; MGMT; slot-blot assay

Keywords Plus: O-6-METHYLGUANINE-DNA METHYLTRANSFERASE; ALKYLATION DAMAGE; REPAIR; DEPLETION; EXPOSURE; GENOME; MGMT

Author Information Corresponding Address: Povey, Andrew C. (corresponding author)

Univ Manchester, Fac Biol Med & Hlth, Sch Hlth Sci, Epidemiol & Publ Hlth Grp,Div Populat Hlth Hlth Se, Manchester M13 9PL, England

E-mail Addresses :

apovey@manchester.ac.uk
Addresses :
[1] Univ Manchester, Fac Biol Med & Hlth, Sch Hlth Sci, Epidemiol & Publ Hlth Grp,Div Populat Hlth Hlth Se, Manchester M13 9PL, England
[2] Manchester Univ Fdn Trust, Wythenshawe Hosp, Prevent Breast Canc Ctr, Manchester M23 9LT, England
[3] Christie NHS Fdn Trust, Manchester Breast Ctr, Wilmslow Rd, Manchester M20 4GJ, England
[4] Univ Manchester, Fac Biol Med & Hlth, Manchester Acad Hlth Sci Ctr, Div Canc Sci,Sch Med Sci, Manchester M13 9PL, England
E-mail Addresses :
hanumyaakub@iium.edu.my; anthony.howell@manchester.ac.uk; gmargison@manchester.ac.uk; apovey@manchester.ac.uk

Data availability statement

The data presented in this study are available on request from the corresponding author.

Categories/ Classification

Research Areas: Environmental Sciences & Ecology; Toxicology

Citation Topics: 1 Clinical & Life Sciences › 1.152 Molecular & Cell Biology - DNA Damage › 1.152.251 Nucleotide Excision Repair

Sustainable Development Goals: 03 Good Health and Well-being

Web of Science Categories

Environmental Sciences; Toxicology

Funding

View funding text

Funding agency	Grant number	
Ministry of Education, Malaysia		Hide details
Appeared in source as: Malaysia Ministry of Higher Education		
Malaysia Ministry of Higher Education and International Islamic University Malaysia		
Cancer Research UK		Show details
European Union (EU)		Show details

Show All Details

Language	English
Accession Number	WOS:001326227900001
PubMed ID	39330577
eISSN	2305-6304
IDS Number	H8Y1J

– See fewer data fields

Journal information

TOXICS

eISSN	2305-6304
Current Publisher	MDPI, MDPI AG, Grosspeteranlage 5, CH-4052 BASEL, SWITZERLAND
Research Areas	Environmental Sciences & Ecology; Toxicology

0.88
Journal Citation Indicator ™ (2024)

Web of Science Categories	Environmental Sciences; Toxicology

Citation Network

In Web of Science Core Collection

0 Citations

Create citation alert

33
Cited References

→ View Related Records

How does this document's citation performance compare to peers?

← Open comparison metrics panel

Data is from InCites Benchmarking & Analytics

Use in Web of Science

0	0
Last 180 Days	Since 2013

Learn more →

This record is from:

Web of Science Core Collection

- Science Citation Index Expanded (SCI-EXPANDED)

Suggest a correction
If you would like to improve the quality of the data in this record, please Suggest a correction

33 Cited References → View as set of results

Explore