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

Toxics • Article • Open Access • 2024 • DOI: 10.3390/toxics12090649

Yaakub, Hanum [a] ; Howell, Anthony [b, c, d] ; Margison, Geoffrey P. [a] ; Povey, Andrew C. [a]

[a] Epidemiology and Public Health Group, Division of Population Health, Health Services Research and Primary Care, School of Health Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, M13 9PL, United Kingdom

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 µM TMZ and the extracted DNA, analysed using the ASB, was found to contain 1.34 fmoles O^6 -meG/µg 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/µg DNA. This study shows the potential usefulness of the ASB assay to detect and quantify total O^6-AlkGs in human DNA samples. © 2024 by the authors.

Author keywords

Atl1; MGMT; N-nitroso compounds; O^6-alkylguanines; slot-blot assay

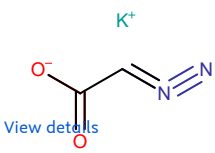
Indexed keywords

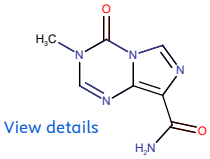
EMTREE drug terms

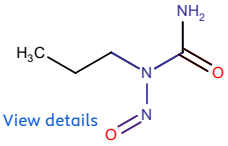
6 o alkylguanine DNA alkyltransferase; methylated DNA protein cysteine methyltransferase; temozolomide

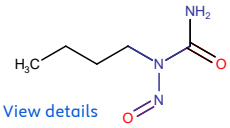
EMTREE medical terms

alkylation; Article; breast tumor; controlled study; DNA repair; human; human cell; human tissue; in vitro study; MCF-10A cell line; placenta; Schizosaccharomyces pombe; screening


View details


View details


View details


View details


View details

Powered by Reaxys

Chemicals and CAS Registry Numbers

Unique identifiers assigned by the Chemical Abstracts Service (CAS) to ensure accurate identification and tracking of chemicals across scientific literature.

methylated DNA protein cysteine methyltransferase

temozolomide	85622-93-1

Corresponding authors

Corresponding author	A.C. Povey
Affiliation	Epidemiology and Public Health Group, Division of Population Health, Health Services Research and Primary Care, School of Health Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, M13 9PL, United Kingdom
Email address	apovey@manchester.ac.uk

About Scopus

Language