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Widespread distribution of DNA glycosylases removing oxidative DNA lesions in human and rodent brains

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ABSTRACT

High metabolic activity and low levels of antioxidant enzymes make neurons particularly prone to damage by reactive oxygen species. Thus, repair of oxidative DNA damage is essential for normal brain function. Base excision repair is the major pathway for repair of oxidative DNA damage, and is initiated by DNA glycosylases recognizing and removing the damaged base. In mammalian cells at least five different DNA glycosylases with overlapping substrate specificity, NEIL1, NEIL2, NEIL3, OGG1 and NTH1, remove oxidative DNA base lesions. Here we report mRNA expression and distribution of these five DNA glycosylases in human and rodent brains using *in situ* hybridization and Northern blotting supported by glycosylase activity assays. *NEIL1*, *NEIL2*, *OGG1* and *NTH1* showed widespread expression at all ages. *In situ* hybridization studies in mouse brain showed that expression of *mNeil1* increased with age. In newborn mouse brain, *mNeil3* revealed a discrete expression pattern in brain regions known to harbour stem cell populations, i.e., the subventricular zone, the rostral migratory stream, and the hilar region of the hippocampal formation. Expression of *mNeil3* decreased with age, and in old mice brains could be detected only in layer V of neocortex. *MNth1* was constitutively expressed during lifespan. In Northern blots, *mOgg1* expression showed a transient decrease followed by an increase after 8 weeks of age. Assays for faPy DNA glycosylase activity revealed increased activity level with age in all brain regions analyzed.

The widespread but differential expression of the DNA glycosylases recognizing oxidative base lesions suggests distinct and age dependent roles of these enzymes in genome maintenance in brain. The distribution of *mNeil3* is particularly intriguing and points to a specific role of this enzyme in stem cell differentiation.

Abbreviations: 8-oxoG, 8-oxoguanine; AP, apurinic/apyrimidinic; BER, base excision repair; CP, caudatoputamen; CS, Cockayne syndrome; DAR, differentiation-associated repair; faPy, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; GGR, global genomic repair; NEIL, *Escherichia coli* endonuclease VIII-like; NTH, Endonuclease III; OGG, 8-oxoG-DNA glycosylase; P3, postnatal day 3; RMS, rostral migratory stream; ROS, reactive oxygen species; SVZ, subventricular zone; TCR, transcription-coupled repair.

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1. Introduction

A common feature of neurodegenerative diseases, as well as aging, is elevated levels of DNA damage [1,2]. A major cause of DNA damage is oxidative stress, which contributes to cell death in stroke and chronic conditions such as Alzheimer's and Parkinson's diseases [2–6]. Neurons are continuously challenged by reactive oxygen species (ROS), which may damage nucleic acids and induce cell death. Thus, effective neuronal repair of oxidative damage is critical to genome maintenance and brain function [2].

Base excision repair (BER) is the main pathway for repair of oxidative DNA lesions. BER is initiated by damage-specific DNA glycosylases, which remove the modified base, leaving an abasic site which is acted upon by an intrinsic AP lyase activity (bifunctional glycosylase) or by an AP endonuclease. In the resulting strand break, lyases and/or nucleases remove the sugar-phosphate backbone, and DNA polymerases and ligases fill and reseal the gap [7]. Five mammalian DNA glycosylases excising oxidized DNA bases have been described: NEIL1, NEIL2, NEIL3, OGG1 and NTH1.

HNEIL1 removes a number of oxidative lesions including 8-oxoguanine (8-oxoG), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (faPy) and thymine glycol [8–10]. Embryonic mouse stem cells deficient in mNeil1 are sensitive to low levels of γ-radiation, indicating that mNeil1 is important for the cellular defence against DNA damage [11].

HNEIL2 mainly cleaves oxidized pyrimidine substrates from double stranded DNA, also in bubble-structured DNA [12]. MNeil1 and mNeil2 revealed unique specificity towards spiroiminodihydantoin and guanidinohydantoin lesions, which are more mutagenic, oxidized products of 8-oxoG [13].

HNEIL3 possesses a weak DNA glycosylase activity against faPy in crude insect cell extracts [8]. Moreover, mice deficient in *mNeil3* exhibit no apparent phenotype [14].

HOGG1 primarily removes oxidized purines as 8-oxoG and faPyG [15–19], whereas hNTH1 excises oxidized pyrimidines, including thymine glycol, 5-hydroxycytosine, dihydrothymine and dihydrouracil [20,21]. The mild phenotypes associated with targeted disruption of the mouse *mOgg1* and *mNth1* genes indicate existence of backup enzymes for the repair of these lesions, such as the NEIL enzymes.

There is scant knowledge about oxidative DNA repair in neurons. Several of the BER enzymes have been shown to be expressed in the brain, but distribution of DNA glycosylases in brain has been published only for *Ogg1* [22].

We have made extensive tests of commercial and custom-made antibodies to DNA glycosylases and none of these antibodies show sufficient selectivity. Notably, antibodies to OGG1 produce staining even in animals with targeted disruption of this gene. Pending antibodies with adequate selectivity and affinity, localization studies of DNA glycosylases cannot be performed by immunocytochemical approaches. Here we have used Northern analysis and *in situ* hybridization to describe the distribution of mRNAs of the five different DNA glycosylases, *NEIL1*, *NEIL2*, *NEIL3*, *OGG1* and *NTH1*, in mammalian brains. Finally, we examined the transcript distribution and faPy DNA glycosylase activity during development and aging.

2. Methods

2.1. Northern blot hybridization

Northern blots containing multiple adult human brain tissue samples purchased from Clontech (catalog numbers 636802 and 636838) were probed with full-length DNA probes of *hNEIL1*, *hNEIL2*, *hNEIL3*, *hOGG1* and *hNTH1* expression. Mouse brain aging blot (MBAB 1009-1) was purchased from Seegene Inc. (Soul, Korea), and probed with full-length mouse *Neil1* and *Neil3*. *Ogg1* probe containing exon 4–7, *Nth1* probe including exon 4–6 and *Neil2* probe of exon 2 was utilized. Northern blot hybridization was carried out using ExpressHyb solution (Clontech) as recommended by the manufacturer. Probes were labelled with (α-^{32}P)-dCTP using the Rediprime DNA labelling system (Amersham Corp.).

2.2. Animals

Brains from C57Bl/6J mice were used. Mice were housed and handled in accordance with the European Council Directive 86/609/EEC.

2.3. Tissue preparation

Mice at postnatal day 3 (P3), 1 month or 1 year of age were decapitated and the brains carefully removed. The brains were quickly frozen on dry ice and stored at −80 °C. Horizontal or sagittal sections were cut at 15 μm on a cryostat and mounted onto slides (Super frost). The sections were post-fixed in 4% formaldehyde and stored in 96% ethanol at 4 °C until use.

2.4. Probe synthesis

Full-length mouse *Neil1* DNA (IMAGE clone 1399170) was cloned into pT7T3D-Pac vector with restriction enzymes Not1 and EcoRI, and linearized with BspHI. MNeil2 exon2, mNeil3 full-length and mNth1 exon 4–6 were cloned into pT7T3-Pac vector with the restriction enzymes Not1 and EcoRI. MNth1 was linearized with FspHI and mNeil2 and mNeil3 were linearized by BspHI. MOgg1 containing exon 4–7 was cloned into Topo Zero blunt vector. All DNA templates were purified by GFX column (Amersham) before transcribing RNA digoxigenin (DIG) probes by DIG-RNA Labelling kit (Roche). Both antisense and sense probes for each gene was generated (for details see [23]). The RNA probes were tested on agarose gel, and the quality of DIG incorporation was tested by dot blot according to DIG nucleic acid detection kit (Roche).

2.5. In situ hybridization

Horizontal and sagittal sections of mouse brain aged P3, 1 month or 1 year were labelled with full-length DIG-RNA antisense probes against *mNeil1* or *mNeil3*. For *mOgg1* a probe with exon 4–7 was used, for *mNeil2* a probe with exon 2 and