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Research article

Expression of nucleotide excision repair in Alzheimer's disease is higher in brain tissue than in blood

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ABSTRACT

Age-related changes are increased in patients with Alzheimer's disease (AD), including oxidative stress and DNA damage. We propose that genotoxic stress and DNA repair responses influence neurodegeneration in the pathogenesis of AD. Here, we focus on nucleotide excision repair (NER). Real-time qPCR and mass spectrometry were employed to determine the expression levels of selected NER components. The mRNA levels of the genes encoding the NER proteins RAD23B, RPA1, ERCC1, PCNA and LIG3 as well as the NER-interacting base excision repair protein MPG in blood and brain tissue from four brain regions in patients with AD or mild cognitive impairment and healthy controls (HC), were assessed. NER mRNA levels were significantly higher in brain tissue than in blood. Further, LIG3 mRNA levels in the frontal cortex was higher in AD versus HC, while mRNA levels of MPG and LIG3 in entorhinal cortex and RPA1 in the cerebellum were lower in AD versus HC. In blood, RPA1 and ERCC1 mRNA levels were lower in AD patients than in HC. Alterations in gene expression of NER components between brain regions were associated with AD, connecting DNA repair to AD pathogenesis and suggesting a distinct role for NER in the brain.

1. Introduction

Although Alzheimer's disease (AD) has been recognized since the beginning of the 20th century, we still have little information about the etiology at the molecular level. One of the main challenges in AD research is to define biomarkers with high sensitivity and specificity that are present in the pre-symptomatic stages of the disease, so that intervention can be initiated at an early stage. An optimal test would be a set of biomarkers present in a blood sample. However, in order to achieve this, we need more knowledge on the etiology of early AD.

Aging is the major risk factor for the development of AD, and age-related changes are increased in AD and mild cognitive impairment (MCI), including DNA damage and oxidative stress [2,3,5,10,16,25,26]. We propose that an imbalance in oxidative stress and DNA repair responses influence neurodegeneration in AD. DNA damage is repaired by several DNA repair mechanisms, including nucleotide excision repair (NER) and base excision repair (BER).

The NER pathway repairs bulky helix-distorting damage events in DNA, which can be caused by several various sources including UV radiation, chemical adducts and oxidative stress. NER is one of the most well-characterized mechanisms of DNA repair, and defects in the NER pathway are recognized causes of several neurodegenerative diseases, such as Cockayne syndrome (CS), trichothiodystrophy and some subtypes of xeroderma pigmentosum (XP) [6,17]. The association between NER and other neurodegenerative disorders in humans is, however, poorly understood [21]. It is proposed that NER-inflicted neurodegeneration most likely is caused by endogenous DNA lesions [1], since exogenous stress such as UV radiation does not directly affect the brain and most chemical adducts do not cross the blood-brain barrier. Endogenous DNA lesions are most commonly caused by reactive oxygen species (ROS) and as a consequence, damaged macromolecules including DNA are generated.

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The NER pathway involves more than 30 enzymes and is separated into two different pathways based on the manner of damage recognition [11]. The transcription coupled repair pathway (TC–NER) repairs DNA lesions that are detected through the blockade of the RNA polymerase II enzyme, while in global genome (GG)-NER, the damage is mostly recognized by the XPC-RAD23B complex [12,21]. RAD23B has been shown to interact with the 3-methyladenine DNA glycosylase (MPG) of the BER-pathway and elevates glycosylation of MPG-specific DNA damages [14], suggesting its effect in damage recognition in both NER and BER.

The remaining part of the NER repair pathway is common for both sub-pathways. The damaged DNA helix is unwound by helicases in the transcription factor II H-complex (TFIIH). The Replication Protein A (RPA)-complex, with the subunits RPA1-3, binds to the undamaged DNA strand. Incisions in the damaged DNA strand are made by XPG and the XPF-ERCC1-complex, respectively, in the 3′ and 5′ sites of the damage event. This results in the release of an oligomer of 27–30 nucleotides [21]. The remaining gap is filled by the DNA polymerase subunits δ, κ and ε, which are recruited by the PCNA clamp, and is finally sealed by either LIG1 or the XRCC1-LIG3-complex [11,21].

The BER pathway is the predominant DNA repair pathway for the processing of small base lesions derived from oxidation and alkylation events [20]. Multiple studies show altered BER profiles in the prodromal phases of AD in both mice [9,15,22] and human brain tissue [8,10,25]. Less is, however, known about NER in human neurodegenerative disorders [21]. Although mice lacking NER components such as ERCC1, XPF and other enzymes related to XP or CS have provided good models for neurodegeneration [17], their potential role in explaining the etiology of AD still remains unclear.

2. Materials and methods

2.1. Ethical statement

The study was approved by the Norwegian Regional Committee for Ethics in Medical Research (REK 2013/1643 and REK 2011/698). Written, informed consent was obtained from all participants. Donation, storage and transfer of human brain specimens were also approved by the external Research Ethical Committee of the Fundacion Centro de Investigación de Enfermedades Neurológicas (CIEN) biobank (Research Ethical and Animal Welfare Committee, ISCIII, Spain).

2.2. Brain specimens and blood samples

Freshly frozen post-mortem brain tissue specimens from a cohort of 43 AD patients and 9 healthy controls (HC) were harvested by CIEN. Specimens from the frontal cortex (FC), cerebellum (CB), entorhinal cortex (EC) and the hippocampus (HCP) were investigated, representing a total of 157 brain specimens from 43 AD patients and 33 brain specimens from 9 HC (Table S1). For further details, see Additional file 1 in Lillenes et al. [8].

Blood samples from 51 AD patients, 24 MCI patients and 62 HC were collected in PAXgene tubes at the Memory Clinic at Oslo University Hospital (Ullevål) [23]. AD patients were diagnosed to have suspected AD according to the NINCDS-ADRDA criteria [13], while patients with MCI had to fulfill either the ICD-10 criteria or the Winblad criteria for MCI [27]. Patients with frontotemporal, Lewy-Body and vascular dementia, as well as those with severe depression or psychotic features, were excluded from the study. All samples were immediately stored at −80 °C until further use.

2.3. RNA isolation

For information on RNA isolation and determination of RNA concentration, please see the Supplementary Material.

2.4. Quantitative real-time PCR

Reverse transcription and quantitative real-time PCR (qRT-PCR) was performed using TaqMan Gene Expression assays for the RAD23B (HR23B), RPA1, ERCC1, PCNA, LIG3, and MPG mRNAs (Table S2). Glyceraldehyde phosphate dehydrogenase (GAPDH) was selected as the reference gene after validating 32 candidate genes as internal control for all four brain regions and blood samples in both AD and HC, using TaqMan Human Endogenous Control Plates (Applied Biosystems, Foster City, California, USA). For further details, see the Supplementary Material and Additional file 1 in Lillenes et al., 2016 [8].

2.5. Proteomic analysis by mass spectrometry

Proteomic analysis was performed using high-resolution mass spectrometry (Q-Exactive, Thermo-Fisher). For further information regarding protein sample preparation and analysis, please see the Supplementary Material.

2.6. Statistical analysis

To statistically test the differences of mRNA levels in blood and brain tissue, a two-sample *t*-test was performed (Table 1, S3, S4). The level of significance was adjusted using the Bonferroni correction.

The mRNA levels in blood in the AD, MCI and HC groups were statistically compared using a one-way ANOVA analysis (Table 2). If the F-test p-value was less than 0.05, Tukey's test was used for pairwise comparisons which controls the family-wise error rate [24]. A linear mixed model was used to test the differential mRNA levels between AD and HC in the four human brain regions for each of the six genes (Table 3). The fixed effects in the model were disease, brain region and the interaction between disease and brain region. Potential correlation between brain regions from the same patient was corrected for using a random individual effect. An F-test was used to monitor the significance of the overall interaction between brain part and disease status. When the overall interaction effect was non-significant, the significance of the disease effect could be assessed directly using a *t*-test. If the interaction effect was significant, a reduced mixed model was used to test the interactions separately for each brain part. This model included fixed effects for brain region, interaction between brain region and disease status and the random effect. To test the interaction of disease status and brain part, a *t*-test was used with a significance level of 0.05.

3. Results

3.1. Higher NER mRNA levels in brain tissue than in blood

We compared mRNA levels from brain tissue and blood samples from AD patients and HC (Table 1). mRNA levels for RAD23B, RPA1, ERCC1, PCNA, LIG3 and MPG were monitored (Fig. 1). The most

Table 1
mRNA levels of RAD23B, LIG3, ERCC1, RPA1, PCNA and MPG in brain and blood.

Gene	Mean GE in blood	Mean GE in brain	p-value	Mean GE in blood	Mean GE in brain	p-value
	Alzheimer's disease			Healthy controls		
RAD23B	0,80	1,26	1,77E-09 *	0,88	1,27	6,00E-03
RPA1	0,73	1,54	< 2,2E-16 *	0,88	1,69	8,00E-04 *
ERCC1	0,58	2,42	< 2,2E-16 *	0,67	2,04	7,70E-06 *
PCNA	0,85	1,50	9,00E-08 *	0,94	1,24	7,10E-02
LIG3	0,48	2,58	< 2,2E-16 *	0,51	2,44	1,30E-07 *
MPG	0,92	1,20	6,90E-05 *	1,01	1,27	4,00E-02

*denotes significant results after Bonferroni-correction.