When parsimony backfires: neglecting DNA repair may doom neurons in Alzheimer's disease

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Summary

Taking advantage of the fact that they need not replicate their DNA, terminally differentiated neurons only repair their expressed genes and largely dispense with the burden of removing damage from most of their genome. However, they may pay a heavy price for this laxity if unforeseen circumstances, such as a pathological condition like Alzheimer's disease, cause them to re-enter the cell cycle. The lifetime accumulation of unrepaired lesions in the silent genes of neurons is likely to be significant and may result in aborting the mitotic process and triggering cell death if the cells attempt to express these dormant genes and resume DNA replication.

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Introduction

Alzheimer's disease (AD) is one of the main sources of dementia in our society: roughly one person in five will suffer from AD by the age of 80. Anatomically, the disease is characterized by regional loss of neurons in various areas of the brain, resulting notably in cortical atrophy in the temporal lobes and hippocampal regions. Microscopically, the hallmarks of AD are (1) neurofibrillary tangles, an intracellular accumulation of paired helical filaments, and (2) senile plaques, depositions of protein fibers and amyloid peptides, surrounded by a rim of dystrophic neurons and glial cells.[1,2] Mutations in the amyloid precursor protein gene have been detected in a small subset of familial AD patients,[3] prompting the suggestion that amyloid accumulation may cause the disease.[4] However, the precise mechanism(s) for neuronal degeneracy have not yet been elucidated.[2]

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Abbreviations: AD, Alzheimer's disease; BER, base excision repair; DAR, differentiation-associated repair; FISH, fluorescence in-situ hybridization; GGR, global genome repair; NER, nucleotide excision repair; TCR, transcription-coupled repair; UV, ultraviolet light.

Neurons do not repair the bulk of their genome

We and others have observed that terminally differentiated neurons do not efficiently remove DNA damage from the bulk of their genome. Mouse neuroblastoma cells were shown to be more sensitive to ultraviolet-induced lesions[5] and to gamma-induced strand breaks[6] after differentiation than while they were actively proliferating. Human neuroblastoma cells removed bulky DNA adducts less rapidly following differentiation.[7] Chick embryo neurons are much less efficient than fibroblasts in repairing UV-induced lesions,[8] and rat cortical neurons are highly sensitive to X-rays and they repair strand breaks much less rapidly than do astrocytes.[9] Mouse neuroblastoma cells display a gradual decrease in their ability to repair UV-induced lesions as they differentiate.[10] Our studies with human NT2 cells and pre-natal human neurons have documented a similar, striking attenuation in nucleotide excision repair (NER) in differentiated cells (Refs. 11,12, and T.N., unpublished data).

NER is the most versatile DNA repair system: it can handle a wide array of DNA damage, from UV-induced lesions to intrastrand cross-links and bulky chemical adducts. It also acts on some lesions produced by reactive oxygen species, including cyclodeoxyadenosine, thymine glycol and 8-oxo-guanine. Roughly thirty proteins are required to carry out the steps of NER. These include recognition of DNA lesions, excision of a short single-strand segment of DNA spanning the lesion, and filling the resulting gap by repair replication, using the intact complementary strand as a template.[13]

Even though differentiated cells express residual NER activity (as little as 10% for some lesions), it is unlikely that this would be adequate to maintain a lesion-free genome. The load of DNA lesions results from an equilibrium between damage creation and repair. As long as the rate of repair is higher than the rate of damage, the genome can be kept relatively lesion-free. Attenuating repair capacity by tenfold will very likely tilt the balance the other way and lead to a slow and steady accumulation of lesions throughout the genome. It appears that neurons, and likely other terminally differentiated cells, accommodate this situation to dispense with the energy cost of maintaining global DNA repair systems, such as NER. Since these cells will never again replicate or transcribe the bulk of their DNA, accumulating damage should not have any

nefarious consequences. However, after many years, it would be predicted that most of the genes would be crippled by this process.

Then, in order to remain alive and functional, these cells must maintain the integrity of the subset of genes that they are transcribing. We have observed that this is indeed the case in human neurons, in which UV-induced lesions are proficiently removed from active genes, even though the bulk of the genome (including silent genes) is not efficiently repaired.[11] This is probably due to the transcription-coupled repair (TCR) pathway, which is known to target NER enzymes to the transcribed strand of active genes, most likely by using translocating RNA polymerase II as a lesion sensor.[14,15] Surprisingly, we have also observed proficient repair in the non-transcribed strand of active genes in neurons, and we are currently studying this phenomenon that we have named "differentiation-associated repair" (DAR).[11] Although we do not yet know the mechanistic details of DAR, it makes sense that a dedicated system might exist to maintain the integrity of the non-transcribed strand in active genes, since TCR must use that strand as a template to repair damage in the transcribed strand.

Neurons in Alzheimer's disease re-enter the cell cycle before they die

A growing body of evidence suggests that the neurons that are about to die are those that have attempted to re-enter the cell cycle and synthesize DNA. It was reported that, shortly before cell death, cell-cycle-related proteins are unexpectedly expressed in the neurons of Alzheimer patients. An increase of the proliferation-associated antigen Ki-67 was observed by several research groups.[16,17] More recently, several laboratories have reported similar results with various cell-cycle-related proteins: (1) the Cdc2 kinase and its regulator cyclin B1,[18] (2) the cyclin-dependent kinase Cdk-4 and its inhibitor p16,[19] (3) Cyclin D, Cdk-4, cyclin B1 and the proliferating cell nuclear antigen (PCNA),[20] (4) the "polo-like" kinase,[21] and (5) Cdc25A, a phosphatase that activates Cdc2.[22]

Of course, this indirect evidence does not constitute formal proof that neurons do re-enter the cell cycle. It is conceivable that the aberrant expression of cell-cycle control factors may have no effect in a terminally differentiated cell. However, in a very elegant piece of work, Yang et al.[23] have recently demonstrated by fluorescence in-situ hybridization (FISH) that genomic DNA is indeed replicated in some neurons of Alzheimer patients. The authors monitored four different loci in three autolog chromosomes, in autopsy material from Alzheimer patients. In roughly 4% of the neurons they observed four fluorescent spots for each gene, instead of the two expected. This provides strong evidence that these neurons had replicated their DNA. There were some differences among the various brain areas: in the hippocampus the spots were scattered, whereas in the basal nucleus they were observed in pairs, perhaps indicative of a problem with the cohesin complex that normally holds chromatids together during chromosome replication. These authors also observed an induction of the G_2-phase-specific protein, cyclin B, suggesting that the neurons were able to proceed beyond the S phase, into G_2.

This phenomenon is not unique to AD neurons

Interestingly, neurons have also been shown to express cell-cycle-related proteins in other neurodegenerative diseases. For example, Smith and Lippa[16] also demonstrated an increase in Ki-67 in the brains of patients with Pick's disease, progressive supranuclear palsy, Lewy body disease, and Parkinson's disease, as well as in ganglioma, and even in the aging brain. Nagy et al.[17] also observed increased Ki-67 levels in Parkinson's disease and in Down's syndrome. More recently, Husseman et al.[24] observed increased expression of cdc2, cyclin B1 and three cdc2-produced phosphoepitopes in Down's syndrome, frontotemporal dementia linked to chromosome 17, progressive supranuclear palsy, corticobasal degeneration, Parkinson-amyotrophic lateral sclerosis of Guam, Niemann-Pick syndrome type C, and Pick's disease.

Although it is not clear why such diverse pathological conditions should cause terminally differentiated neurons to re-enter the cell cycle, it would appear that such an event is generally fatal for these cells. That fact was experimentally confirmed in principle with model systems in which otherwise normal neurons re-enter the cell cycle. Several groups have utilized the SV40 large T-antigen under control of a tissue-specific promoter to neutralize the Rb protein in a specific cell type. Al-Ubaidi et al. used the opsin promoter to target the rod photoreceptors. In these cells, they observed increased levels of PCNA and thymidine kinase,[25] DNA synthesis, mitotic figures and finally cell death.[26] Similarly, targeting the large T-antigen to cerebellar Purkinje cells resulted in cell death rather than tumoral proliferation.[27] Herrup et al.[28] made use of a mouse mutant with spontaneous defects in cerebellar Purkinje cells, resulting in death of the cerebellar granule cells and the inferior olive neurons. In the neurons of these mice, the authors observed incorporation of the thymidine analogue, 5-bromodeoxyuridine, an indication that DNA replication was occurring. The same cells subsequently died, within about 10 hours, by a process other than the classical apoptosis pathway, as judged by the TUNEL assay. There was also an increase in the levels of PCNA and cyclin D1. Interestingly, these same phenomena were also observed at very low levels in the brains of wild-type mice.

Accumulated DNA damage may kill neurons that change their gene expression pattern

Taken together, the above results indicate that neurons are unable to successfully resume proliferation. If a pathological or

artificial condition causes them the re-enter the cell cycle, they die shortly thereafter. The reason for their demise has not yet been determined, although several hypotheses have been suggested.[28] We propose that neurons are killed by damage accumulated in their dormant genes during their post-mitotic state over many years (Fig. 1).

Even though neurons transcribe a large number of genes, these account for only a small portion of the genome. Upon resumption of the cell cycle, a different subset of genes will become active, including but not limited to cell-cycle-control genes. As most of these genes were previously dormant, they will have accumulated DNA lesions. We propose that it is the persistence of lesions in these silent genes that results in cell death when neurons attempt to re-enter the cell cycle. There could be various reasons for this fatal outcome (Fig. 2):

(1) Resuming the cell cycle will result in transcribing a new subset of genes that were previously dormant, and have not been repaired for years. RNA polymerase II will then encounter a number of lesions within these genes, with different consequences depending on the nature of the lesions. Some lesions arrest translocation of RNA poly-

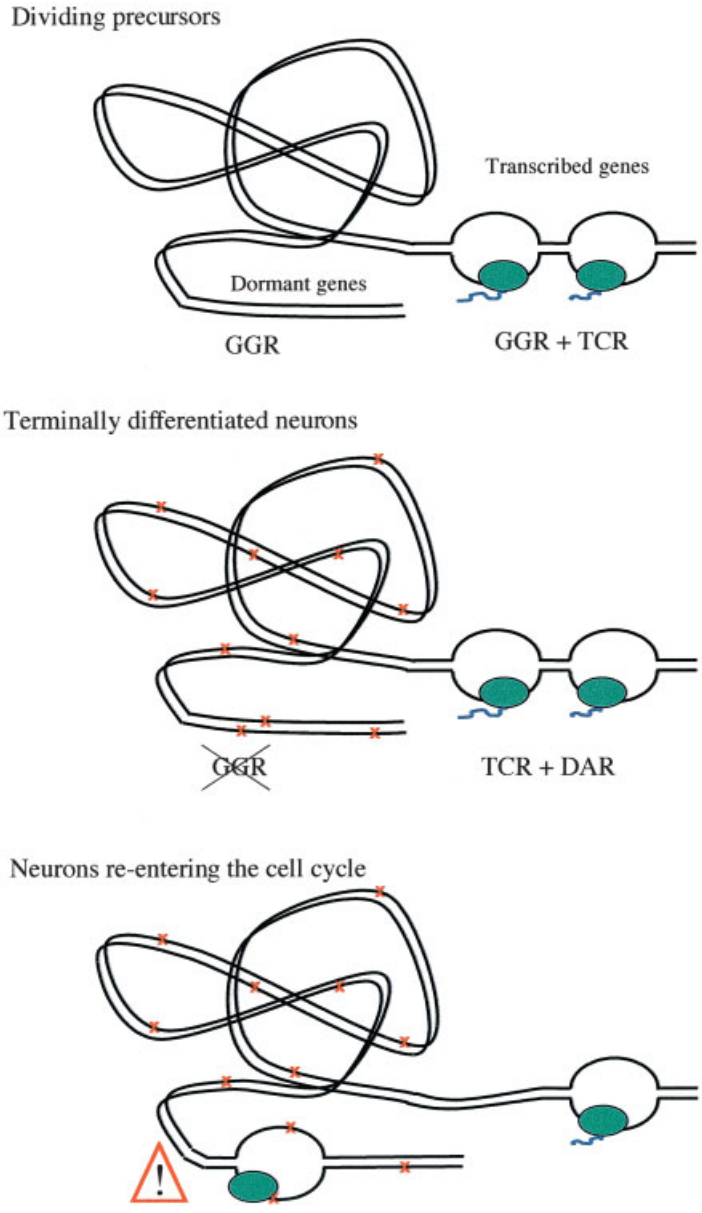

Figure 1. Top: In dividing cells, nucleotide excision repair maintains the integrity of the genome via global genome repair (GGR). In addition, transcription-coupled repair (TCR) targets repair enzymes to the transcribed stand of active genes with higher priority. **Middle:** In terminally differentiated cells, global genome repair is attenuated and lesions accumulate in the bulk of the genome. Active genes, however, are still repaired via TCR and differentiation-associated repair (DAR). **Bottom:** If such cells attempt to re-enter the cell cycle, previously silent genes will be activated. Since they were left unrepaired for years they contain many lesions that are likely to stall RNA polymerase II and lead to apoptosis.

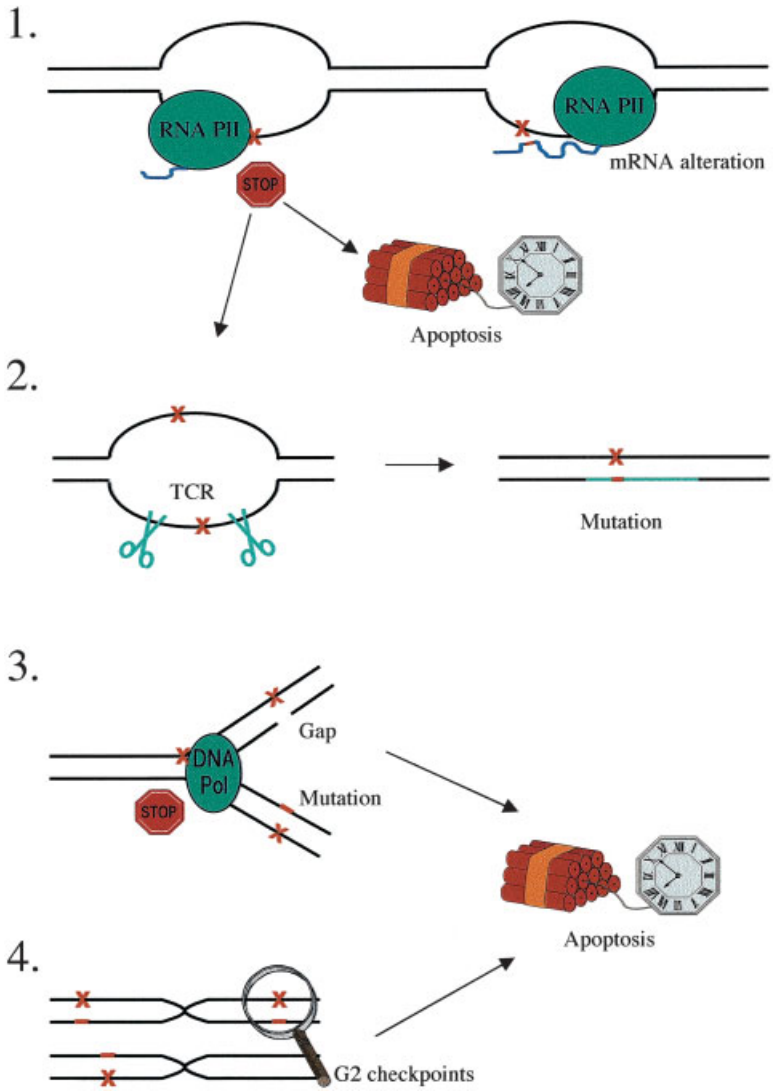

Figure 2. Four possible reasons why accumulated DNA damage may be fatal to neurons re-entering the cell cycle. See text for discussion, the numbers in the figure correspond to the numbered paragraphs. **(1)** RNA polymerase II can be stalled at a lesion, a strong signal for apoptosis, or transcribe through it, potentially yielding defective proteins. **(2)** If two lesions on opposite strands are close enough, transcription-coupled repair will result in introducing mistakes into the transcribed strand. **(3)** DNA damage may impair replication, and trigger apoptosis. **(4)** Previously dormant genomic survey mechanisms may react to the heavily damaged DNA by triggering apoptosis.

merase II, which constitutes a strong signal for apoptosis.[29] Other lesions can be bypassed by the polymerase, but could generate a mistake in the product mRNA. This so called "transcriptional mutagenesis" is likely to yield to non-functional and/or unstable proteins, which will greatly impair the function of the cell, possibly leading to its demise.[30,31]

(2) Even though TCR will immediately attempt to restore the original transcribed strand in such genes, to do so it must use the non-transcribed strand as a template and this strand will also have accumulated lesions over the years, (roughly as many as the transcribed strand). The repair process is thus likely to result in the introduction of mutations, thereby crippling the affected genes beyond any possibility of repair.

(3) The presence of many lesions might also cause a failure of DNA replication. However, this is not very likely, since several DNA polymerases are known to by-pass DNA lesions.[32] Also, the FISH results of Yang et al.[23] indicate that DNA replication is successfully completed prior to cell death, although this is admittedly a crude assay, as the fidelity of replication and the integrity of the DNA have not been considered. Furthermore, recent work from the Chun group[33] demonstrated that aneuploidy is quite common in mice neuroblasts and even in the brain of adult animals. Thus, it is unlikely that incomplete or faulty DNA replication in itself would be a strong signal for apoptosis.

(4) Cell death may be triggered by genomic survey mechanisms that monitor the presence of DNA lesions. Although such mechanisms must obviously be at rest in terminally

differentiated neurons that accumulate DNA damage, it is conceivable that they could be re-activated when the cell cycle resumes. In this respect, it is striking that Yang et al. calculated that polyploid neurons survive for many days after replicating their DNA.[23] This suggests that some checkpoint mechanism is holding these cells in G_2 phase, waiting for DNA repair to be completed, which of course never happens in these terminally differentiated cells. Apoptosis may ensue once the cell finally concludes that it is damaged beyond repair and "hits the self-destruct button".

How to test this hypothesis?

Our hypothesis is based on two strong premises: (1) that normal neurons do not repair the bulk of their genome via the NER pathway, and (2) that neurons re-enter the cell cycle before dying in Alzheimer's and in several other neurodegenerative diseases. Both premises have been well established by a number of independent research groups. What is needed is proof of a causal relationship between the deficiency in repair, leading to an accumulation of lesions in the DNA, and the death of the neurons that attempt to transcribe and/or replicate this crippled DNA.

An obvious way to address the question would be to document the accumulation of DNA damage in neuronal DNA, for instance in neurons purified from necropsy samples obtained from patients of various ages. Unfortunately, we do not know which lesions to quantify. For reasons of convenience, the NER deficiency in neurons has been documented using DNA-damaging agents that are not likely to occur under physiological conditions: UV light, which does not penetrate the skull, or chemicals like cis-platinum that are normally not present in the blood flow. The most-likely type of DNA damage in neurons is oxidative damage, but that is mostly repaired by a different system: base excision repair (BER). Even though BER can also be coupled to transcription,[15,34,35] and could thus be attenuated like NER in neurons, this has not been demonstrated. Although it would be possible to examine NER of many kinds of DNA lesions resulting from mutagenic compounds present in food or cigarette smoke, this would be difficult and tedious. It should be noted however, that there are a few oxidative lesions, such as cyclo-deoxypurines, repaired by NER. These would of course be prime suspects to investigate.

Perhaps the best approach would be to use an animal model, preferably one prone to neurodegeneracy, and to challenge it with chronic exposure to low doses of a carefully selected DNA-damaging agent. It should then be possible to document an accumulation of damage in the genome of neurons and other terminally differentiated cells, whereas proliferating cells should maintain their DNA free of damage.

The next step would be to demonstrate that neuronal degeneracy correlates with such an accumulation. There are several transgenic mice that would be suitable for this kind of study: mice with mutations in the APP or PS1 gene that mimic familial Alzheimer disease,[36] or mice with mutations in the SOD1 gene that mimic amyotrophic lateral sclerosis, and mice with mutations in SCA genes that mimic spinocerebellar ataxia.[37]

However, one problem when using mice is that NER is known to be somewhat deficient in rodents, a phenomenon known as the "rodent repairadox".[38] For instance, one of the two main lesions induced by UV light, cyclobutane pyrimidine dimers, is not repaired at the global genome level, due to lack of expression of the p48 regulatory subunit of the NER gene XPE.[39,40] One should thus be careful when selecting a DNA damaging agent for the type of studies outlined above. If UV light is used for reasons of convenience, one should focus on the repair of (6-4)pyrimidine-pyrimidone photoproducts rather than on cyclobutane pyrimidine dimers.

Ideally one would also need an in vitro system, in which terminally differentiated cells (not necessarily neurons) could be induced to resume the cell cycle. It would thus be possible to challenge these cells with DNA-damaging agents prior to de-differentiating them and verify whether impaired transcription will result in cell death and whether TCR will indeed fix mutations in newly activated genes. For instance, the embryocarcinoma cells used by Rasko et al.[10] might be quite useful, as the mechanisms leading to their differentiation by retinoic acid is well studied and can be finely altered.[41] It has also been shown that NGF withdrawal results in expression of cell-cycle-related genes in cultured neurons,[42] suggesting that these may be another promising system. An alternative possibility would be to use intermittent mitotic cells, like hepatocytes or melanocytes, that retain the ability to proliferate given an appropriate stimulation. However, these cells are likely to have proficient DNA repair systems[12] and it may thus be difficult to accumulate enough damage in their silent DNA without having to expose them to lethal doses of DNA-damaging agents.

Conclusion

There may be yet other ways to tackle the problem and our hope is that this article will stimulate sufficient interest that other investigators will also set out to prove (or disprove) our hypothesis. Whatever the outcome, we feel that important insights into the mechanisms of neurodegenerative diseases may be revealed, with new perspectives on the contribution of DNA damage in these pathologies.

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