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The X-chromosome instability phenotype in Alzheimer's disease: A clinical sign of accelerating aging?

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SUMMARY

Premature centromere division, or premature centromere separation (PCS), occurs when chromatid separation is dysfunctional, occurring earlier than usual during the interphase stage of mitosis. This phenomenon, seen in Robert's syndrome and various cancers, has also been documented in peripheral as well as neuronal cells of Alzheimer's disease (AD). In the latter instances, fluorescent *in situ* hybridization (FISH), applied to the centromere region of the X-chromosome in interphase nuclei of lymphocytes from peripheral blood in AD patients, demonstrated premature chromosomal separation before mitotic metaphase directly after completion of DNA replication in G_2 phase of the cell cycle. Furthermore, and perhaps unexpectedly given the presumptive post-mitotic status of terminally differentiated neurons, neurons in AD patients also showed significantly increased levels of PCS of the X-chromosome. Taken together with other phenomena such as cell cycle re-activation and ectopic re-expression of cyclins and cyclin dependent proteins, we propose that AD is an oncogenic phenotype leading to accelarated aging of the affected brain.

Introduction

The centromere plays a fundamental role in accurate chromosome segregation during mitosis and meiosis in eukaryotes. Centromere functions include sister chromatid adhesion and separation, microtubule attachment, chromosome movement and mitotic checkpoint control [1]. Sequential separation and segregation of centromeres are genetically controlled [2], and this sequence of temporal order is altered in Alzheimer disease (AD), i.e., centromeres prematurely divide in a process known as premature centromere separation (PCS) (Fig. 1). PCS is often regarded as a manifestation of genome instability leading to aneuploidy in aging [2,3], AD [4–6], and other chromosome instability syndromes [7,8].

Primary neurons in the normal brain are viewed as being quiescent and in G_0. However, in AD, multiple lines of evidence suggest that neurons vulnerable to degeneration emerge from this post-mitotic state–phenotypically suggestive of cells that are cycling, rather than in the normal, terminally differentiated, non-dividing state. The successful duplication of DNA [9] indicates that at least some neurons successfully complete S phase. This precludes the possibility that the re-expression of various cell cycle markers is merely an epiphenomena caused by reduced proteasomal activity.

Although various mitotic markers are upregulated in vulnerable neurons in AD, no evidence of actual mitosis has ever been found, suggesting that these neurons are arrested at a point(s) prior to the actual event of cellular division. However, it is well known that once neurons enter S phase, as is the case in neurons in AD [9], the arrested cells lack the ability to return to G_0 and therefore must either complete the cycle or die. Therefore, given the lack of evidence for successful completion of the cell cycle, it is likely that the re-activation of cell cycle machinery in post-mitotic neurons leads to their death. In support of this theory, when a powerful oncogene, SV40 T antigen, is expressed specifically in maturing Purkinje cells in transgenic mice, the cells replicate their DNA (i.e., initiate cell cycle) but then subsequently degenerate and die [10]. Similarly, the expression of SV40 T antigen by the rhodopsin promoter causes photoreceptor degeneration, again associated with cell cycle re-activation and DNA synthesis [11].

Linking PCS with cell cycle changes, PCS appears in the interphase of the cell cycle [12,13]. In initial studies, the FISH method for analysis of the centromere regions of chromosomes 18 and 21 in hippocampal interphase nuclei pointed to the ultimate death of these cells as a consequence of genetic imbalance and

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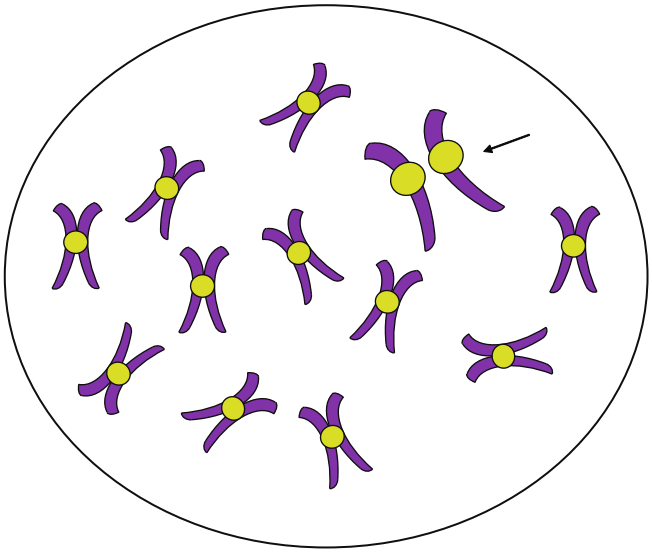

Fig. 1. Premature centromere separation (PCS) demonstrates genomic instability in AD patients. Arrow indicates prematurely separated chromosome with two centromeres, one for each sister chromatid. Surrounding chromosomes are depicted in their normal, undivided states, each with one centromere.

tetraploidy [14]. Results from the PCS research [12,15], moreover, such as the identification of binucleated cells in hippocampus [16] also further revealed a lack of said cells' entry into mitosis. Therefore, it is conceivable that AD hippocampal cells do not pass G_2-M transition, but rather undergo apoptosis following G_2 phase.

In an effort to further identify chromosomal dysfunction in AD, this study probed the characteristic PCS of AD neuronal cells. Using FISH, an analysis of premature centromere division of the X-chromosome was made of slides of neurons from the frontal cerebral cortex, in a group of sporadic AD patients and in age-matched controls [12]. The presence of PCS on the X-chromosome was verified in all analyzed individuals. The group of AD sporadic patients had an average frequency of this alteration of 8.60 ± 1.81% compared to the control group with an average frequency of 2.96 ± 1.20% showing a highly statistical significance ($P < 0.01$). Both peripheral blood lymphocytes and neuronal cells express the PCS, X phenotype in women but not in men. We hypothesize that this is a result of the excess X-chromosome that is inactivated.

Neuronal death in AD exerts accelerating aging of the brain [17], but does the X-PCS trait show us a possibility of accelerated chromosome instability, and thus aging in AD patients?

In other studies, the fluorescent *in situ* hybridization (FISH) method applied to the centromere region of the X-chromosome in interphase nuclei of lymphocytes from peripheral blood in AD patients (Fig. 2) demonstrates that PCS appears well before mitotic metaphase, directly after completion of DNA replication in G_2 phase of the cell cycle [13]. Further, using interphase genetics, statistically significant differences of PCS, X trait are found between women and men, and women with AD expressed an increase in PCS, X trait when compared to age-matched controls [13]. These findings in peripheral cells suggest, like other studies [18], that the AD phenotype is not necessarily restricted to the central nervous system.

Is X-chromosome replication asynchronicity leading to accelerated instability?

One of the distinctive hallmarks of X-chromosome in female mammalian cells is their asynchronous, allocyclic, replication patterns during the S phase. Through FISH analysis of the interphase nuclei of the normal female [19], it has been revealed that the late replicating chromosome X undergoes a process of inactivation that involves a transcription silencing of genes. Importantly, it is now known that not all genes on the inactivated X-chromosome actually succumb to said inactivation; such biological imbalance ultimately translates to imperfect chromosome replication. Loci such

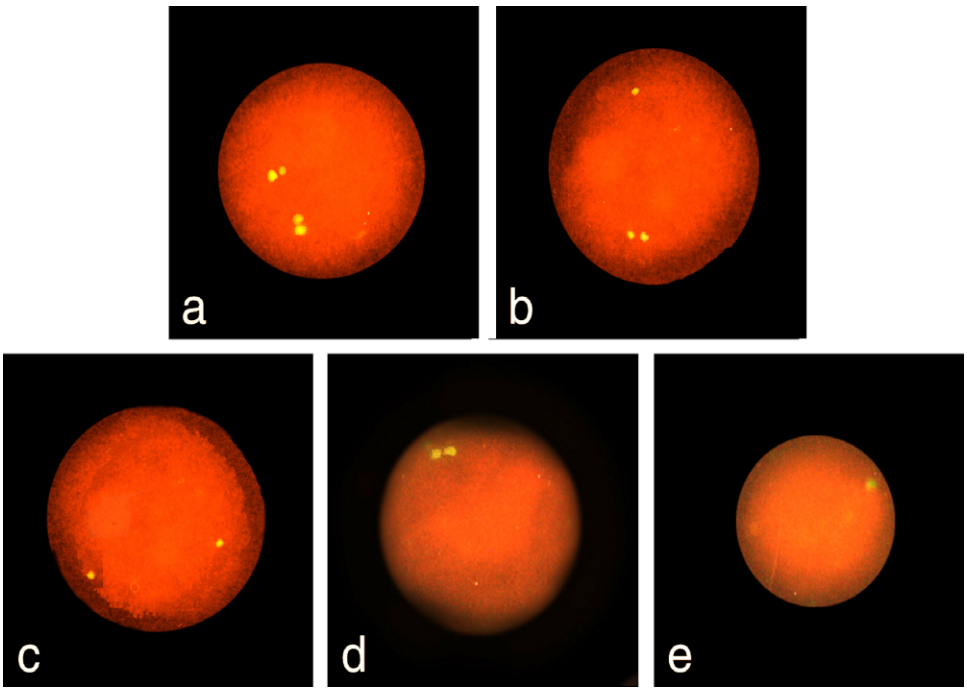

Fig. 2. Fluorescent signals for the centromeric region of chromosome X on interphase nuclei in AD patients and control group. (a) Interphase nuclei in AD female contains two bipartite signals (PCD + on both X-chromosome); (b) interphase nuclei in AD female with a nuclei and one dot like signal (PCD−), and one bipartite signal (PCD+); (c) interphase nuclei of a female from the control group with two separated dot like signals (PCD-); (d) interphase nuclei of a AD male with one bipartite signal (PCD+); (e) interphase nuclei of a male from the control group with one dot like signal (PCD−).

as hypoxanthine–guanine phosphoribosyltransferase (HPRT) and Fragile X-chromosome (FRAXA), for example, which are normally inactivated, display a high degree of replication asynchrony whereas loci that are normally not inactivated, such as ribosomal protein S4, X-linked (RPS4X) and Zinc finger, X-linked (ZFX) are found to replicate very synchronously. Also, the X-inactive-specific transcript gene (XIST) that is expressed from the inactive X-chromosome revealed that it also replicated asynchronously [19,20] with the expressed copy apparently replicating first. Ultimately, it appears that normal versus abnormal X-chromosome homologue replication, separation and segregation is strongly related to the function of time.

In AD, the X-PCS phenotype is accelerated in women. For example, women have twice the frequency of the DXS 1047 202-bp allele (tandem repeat polymorphisms located on the X-chromosome) as men, however the presence of the allele does not affect the age of onset of dementia in either sex [21,22]. Notably, carriers of X-chromosome -linked mutations, such as individuals with Fragile X-associated tremor/ataxia syndrome (FXTAS), demonstrate marked ataxia and neurodegeneration coinciding with increased age [23]. Such results, as well as the aforementioned inadequacies of X-chromosome replication function, show that X-chromosome might have a higher susceptibility to AD than other chromosomes. One possible conclusion is that the inactivated X-chromosome is more susceptible to instability as a direct consequence of replication asynchrony of wrongly activated loci on the inactivated chromosome member.

Discussion

The fact that AD affects twice as many women as men, and that women develop AD mainly after the menopause, indicate that possible hormonal factors may play an important role in the loss of the differentiated phenotype in neurons [24–27].

Replication asynchrony increases in women at risk for having aneuploidy offspring, and produces twice the demonstrated relationship between loss of replication control, centromere dysfunction and predisposition to non-disjunction [28,29]. Moreover, these aspects worsen with time as repeated divisions increase the likelihood of replication dysfunction. For instance, repeated non-disjunction of chromosome X, 18, and 21 by PCS in women clinically normal and who have offspring with Down's syndrome have twice the chance to develop AD [3,30,31]. Interestingly, there is a preferential susceptibility of chromosomes X, 18, and 21 in aged and AD subjects, especially the X-chromosome in women [32]. Also, a recent genome-wide association study provided substantial evidence for an association between genetic variation in the protocadherin 11 gene (PCDH 11) on the X-chromosome and increased late-onset Alzheimer's disease in females [33].

Another possibility for X-chromosome instability is the alteration in the expression of the androgen receptor gene that lies on the X-chromosome. The androgen receptor is implicated in X-chromosome instability by its interconnections to a CDK kinase, $CDK11^{p58}$ [34] and its negative regulation. $CDK11^{p58}$ kinase also plays a crucial role in mitotic progression and is required for the maintenance of sister chromatid cohesion as well as the completion of mitosis in human cells [35]. The androgen receptor can therefore be used to assess deviations in distribution (skewed X inactivation) of the X inactivation pattern.

Conclusion

The dysfunctional centromere separation of the X-chromosome due to aberrant cellular aging and its relation to neuronal survival could clearly be an important consideration in the neurodegenerative cascade of AD. Our hypothesis that alteration of locus inactivation patterns of the X-chromosome may have a fundamental impact on the understanding of neuronal cell cycle re-entry and accelerating aging in AD.

Conflicts of interest/role of the funding source

None of the authors have a financial or personal relationship with other people or organizations that could inappropriately influence this work.

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