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COMMENTARY

Huntington Disease

A Disease of DNA Methylation or DNA Breaks?

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In this issue of *The American Journal of Pathology*, Mollica et al¹ proposed an interesting model to explain the clinical evolution of Huntington disease (HD); some DNA repair genes involved in the mismatch/loop out repair pathways (*BRCA1*, *APEX1*, *RPA1*, and *RPA3*) were shown to be down-regulated in mitotically active HD cells. The authors interpreted their findings by further hypothesizing that DNA methylation is responsible for this DNA repair gene down-regulation and leads to HD trinucleotide repeat region (TNR) instability in HD cells. According to Mollica et al,¹ epigenetics may help understand the specific features of HD cells.

DNA Methylation Is a Common Feature of Neurodegenerative Diseases

R.D. Hotchkiss showed in 1948 that DNA can be methylated at the 5-position of cytosine.² Progressively, the major neurodegenerative diseases were associated with abnormal DNA methylation of some critical genetic loci. However, the exact nature of the link between neurodegeneration and impaired DNA methylation must be discussed. The findings of Mollica et al¹ strengthen the discrepancies of literature: is DNA hypermethylation or hypomethylation associated with neurodegeneration and TNR instability? With regard to neurodegenerative syndromes, the cytidines were shown to be hypermethylated; however, the whole genome is rather hypomethylated in HD cells. In amyotrophic lateral sclerosis cells, the promoter regions of *VEGF* are hypomethylated, whereas those of *ATXN2* are hypermethylated.³ With regard to TNR stability, the DNA methyltransferase inhibitor 5-azacytidine, like cytosine arabinoside, ethidium bromide, and aspirin, increases hypomethylation, and increases the TNR stability from 35% to 75%.⁴ Conversely, the same drug provided clues that hypomethylation increases TNR

instability.⁵ The last report and the observations that: i) demethylation by treatment with 5-azacytidine increases the frequency of sister-chromatid exchange⁶; ii) mutations in several DNA methyltransferases lead to hypomethylation and increase genomic instability⁷; iii) DNA hypomethylation leads to elevated mutation rates,⁷ are in contrast to the findings of Mollica et al¹ obtained with 5-azacytidine. The reasons for these discrepancies are not in the scope of this commentary, but they may concern the choice of cellular models and the endogenous levels of DNA methylation. Nevertheless, all these studies, including the one by Mollica et al,¹ conclude that highly unstable expanded simple sequence repeats may act as sensitive reporters of DNA damage.

DNA Damage in HD

HD is caused by mutations of the huntingtin protein that lead to TNR and generate polyglutamine (polyQ).⁸ PolyQ expansion is associated with oxidative stress. Normal huntingtin is required in the base excision repair synthesis and the mismatch repair pathways that manage base damage and DNA adducts, respectively.⁹ In addition, base damage and DNA adduct repair is important in neurodegenerative

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diseases, as reported for approximately 15 neurological disorders, including HD.¹⁰

However, there are other types of DNA damage, like single-strand breaks and double-strand breaks (DSBs), that may be involved in the progression of the HD disease^{11,12} and more generally in neurological diseases.^{13,14} Radiosensitivity is one specific clinical feature of HD that is mentioned rarely.^{15–20} Defects in base damage and DNA adduct repair are generally associated with photosensitivity, chemosensitivity, and eventually aging, but not necessarily with radiosensitivity.²⁰ Conversely, there is a large body of evidence that unrepaired DSBs are the key elements of radiosensitivity.²¹ Besides, DSB repair defect is also compatible with neurodegeneration and aging.^{22,23} The role of huntingtin protein in DSB repair must be therefore documented.

HD: Are Cycling Cells the Best Cellular Model?

In humans, DSBs are repaired by two major pathways: the nonhomologous end-joining (NHEJ), generally active in G₀/G₁ (quiescent) cells, and the homologous recombination, generally active in S-G₂/M (proliferating) cells. Because most human cells are quiescent (in children and adults), the NHEJ is the major DSB repair pathway.²⁴ This statement is particularly verified in brain cells in which the proliferation rate is limited during life. Consequently, quiescent cells appear to be the most appropriate cellular model to study neurodegenerative disease. DSBs managed by NHEJ can be detected by the nuclear foci formed by the ATM-dependent phosphorylation of the variant histone H2AX (γ -H2AX), easily quantifiable by using immunofluorescence. The γ -H2AX, ATM proteins are therefore natural sensors of the

NHEJ efficiency.²⁵ Conversely, the BRCA1, APEX1, RPA1, and RPA3 proteins, involved in the replication forks, are more active in proliferating cells than in quiescent cells,¹ but are not required for normal NHEJ.²⁶

HD: A Representative Example of a Radiosensitive Disease Caused by Mutations of Cytoplasmic Protein

Whether mutated or not, the human huntingtin protein is mainly cytoplasmic.¹² It is difficult to explain the consequences of a mutated cytoplasmic protein to the DNA surveillance machinery without indirect mechanisms or protein nucleoshuttling. Recently, by using a collection of 117 fibroblast cell lines showing a large spectrum of radiosensitivity, we pointed out that the ATM protein is mainly cytoplasmic in its dimeric form. Oxidative stress induced by ionizing radiation triggers the monomerization of ATM dimers, which facilitates its diffusion in nucleus. Once in nucleus, ATM participates in the recognition of DSB via the phosphorylation of H2AX and triggers DSB repair. In radiosensitive cases, some cytoplasmic proteins can bind to ATM and delay the ATM nucleoshuttling (Figure 1). The slower the ATM nucleoshuttling, the higher the radiosensitivity.^{27,28} Interestingly, a delay in the ATM nucleoshuttling is also associated with genomic instability, spontaneous DNA damage, aging, neurodegeneration, and/or cancer risk.^{27,29} Mutated huntingtin was shown to bind to ATM, likely through its TNR. PolyQ HD cells elicit significant delay in the radiation-induced ATM nucleoshuttling.¹² This mechanistic model was also consolidated by the use of statins and biphosphonates that both accelerate

Figure 1 Model of ATM nucleoshuttling. Significant genotoxic stress (eg, ionizing radiation) induces monomerization of ATM, which facilitates its diffusion in nucleus. Once in nucleus, double-strand breaks (DSBs) are recognized and repaired. In the case of Huntington disease cells, the mutated huntingtin protein binds to ATM, which delays its nucleoshuttling. Consequently, spontaneous DNA breaks and abnormal DNA methylation rate are observed.

the ATM diffusion in nucleus and decrease the radiosensitivity of HD cells.¹² It is noteworthy that similar observations were performed with progeroid cells³⁰ and that the role of cytoplasmic ATM forms was also highlighted for cells from tuberous sclerosis patients.³¹ Finally, the fact that ATM may be essentially cytoplasmic in neurons suggests that the nucleoshuttling model described herein may be exacerbated in brain cells, giving an important mechanistic element to explain the neurodegeneration linked to HD.³² Hence, because HD belongs to the group of radiosensitive syndromes, we proposed a model in which the nucleoshuttling of the ATM kinase is delayed by mutated huntingtin protein in cytoplasm (Figure 1).¹²

Conclusions

Is the ATM nucleoshuttling model compatible with the hypotheses developed by Mollica et al?¹ Interestingly, the ATM-mutated cells are heavily hypomethylated, suggesting that ATM is involved in the methylation process.³³ Furthermore, fibroblasts from immunodeficiency, centromeric region instability, facial anomalies (ICF) syndrome, associated with mutations in the DNA methyltransferase 3b (*DNMT3b*) gene, show a significant delay in the ATM nucleoshuttling (M.L. Ferlazzo, N. Foray, unpublished data). Altogether, these data are consistent with a general model illustrated in Figure 1 in which: i) the mutations of the huntingtin lead to the formation of TNR polyQ expansion, ii) the resulting TNR polyQ domain binds to ATM in cytoplasm, which delays its diffusion in nucleus, iii) the relative absence of ATM in nucleus decreases the capacity of DSB repair, which leads to radiosensitivity in case of exposure to ionizing radiation, iv) the relative absence of ATM in nucleus also favors DNA hypomethylation of the wide genome and overexpression of some DNA repair genes, like those studied by Mollica et al.¹

The findings of Mollica et al¹ are particularly helpful to evaluate the epigenetic features of HD cells. Further investigations are needed to verify the relevance of their data and of the general model illustrated in Figure 1. Particular care should be taken in the choice of cellular model, the quantification of DNA methylation, and the DNA repair gene to be studied.

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