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MOLECULAR MECHANISMS OF NEURODEGENERATION INVOLVING THE EFFECT OF ENVIRONMENTAL POLLUTANTS ON DNA REPAIR ENZYMES

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Abstract — Several works suggest that oxidative DNA damage and alterations of DNA repair enzymes in neuronal cells contribute to the onset of neurodegenerative diseases and neuronal cancer. Cadmium is a common environmental pollutant able to induce oxidative stress leading to the activation of neuronal death pathways and it is also able to inhibit specific DNA repair enzymes of the BER (Base Excision Repair) pathway, the principle cellular way for repairing oxidative DNA damages. One of these enzymes is APE1, a small multifunctional protein that, besides its key role in the BER pathway, has also a redox function important for the control of the intracellular redox state and for the regulation of gene expression. Furthermore, some papers report its contribution both in neuronal protection from oxidative damage and in the development of neurodegenerative diseases. However, the possible involvement of APE1 and of the BER pathway in cadmium-induced neuronal cell damage is still unknown.

Index Terms — MINA, environmental pollutants, neurodegeneration, cadmium

1 BACKGROUND

The role of DNA repair in neuronal cell survival and in response to environmental insults, such as the generation of oxidative stress by heavy metals (i.e. cadmium), is of particular interest since oxidative DNA damage accumulates in human brains and may play a critical role in the pathogenesis of several neurological disorders (i.e. Parkinson's disease, PD, Alzheimer's disease, AD, and ALS)[1-4]. DNA repair, and in particular the Base Excision Repair (BER) pathway, responsible for removing bulk of oxidative

damage, is essential for the maintenance of the genome stability and for the expression of functional proteins preventing cell cycle arrest and cell death. The study of BER enzymes, whose alterations are associated to the onset of neurodegenerative disorders, may help our understanding of how neuronal cells could succumb to neurotoxicity, providing a possible new strategy for preventing such neuronal deterioration. The Apurinic/apyrimidinic Endonuclease 1 / Redox factor 1 (APE1) is an essential enzyme that, in addition to its role in the BER pathway as the major endonuclease responsible for the removal of abasic sites in DNA and RNA, it is also able to redox-regulated several transcription factors during adaptive cellular response to oxidative stress [5]. Alterations in APE1 expression and mutations of its gene have been detected in patients with a variety of neurodegenerative diseases, indicating a possible role of APE1 in neuronal pathophysiology [5]. Interestingly, APE1 contributes to neuronal survival apparently through its DNA repair function and to the maintenance of brain function particularly when challenged with conditions promoting oxidative stress [6-8]. The question remains, however, whether the neuroprotective effects observed are due to APE1 ability to repair DNA or whether also its redox activity may contribute to neuronal survival [9]. Therefore, given the peculiar features of APE1, dissecting the molecular mechanism regulating its protective function in neuronal cells and the processes responsible for cadmium neurotoxicity could be of great impact especially for the translational relevance in the field of molecular medicine.

2 OBJECTIVES

- Understanding of the molecular mechanisms involved in neurodegenerative diseases.
- Understand the molecular mechanisms regulating APE1 protective functions from oxidative stress in neurons particularly involving environmental pollutants;
- Identification of a panel of small compounds able to promote APE1 protective functions in neurons for further translational studies.

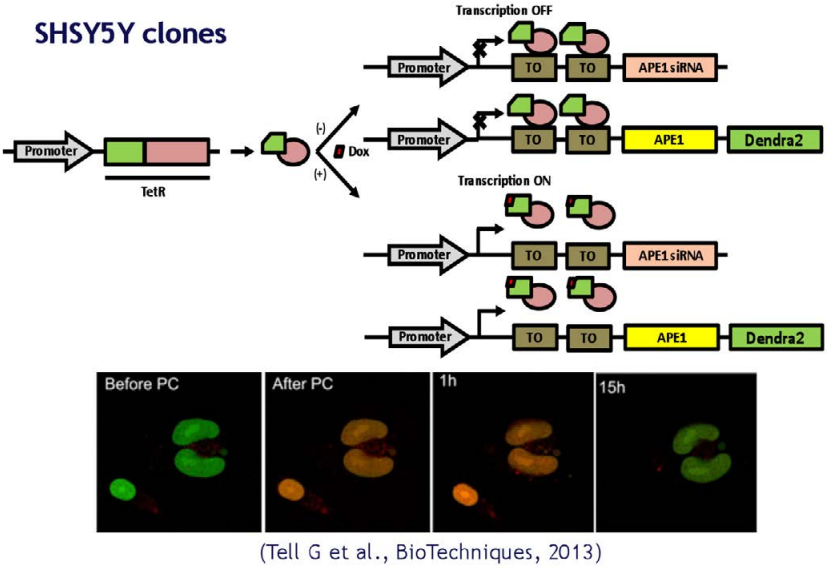
3 APPROACH & METHODS

To address the molecular mechanisms regulating APE1 protective functions from oxidative stress in neuronal cells and, in particular, to dissect which function of APE1 is essential for neuronal protection against environmental stressors (such as cadmium) we decided to generate a neuronal cell model inducibly silenced for APE1 expression. Moreover, to study the APE1 subcellular distribution and its stability upon oxidative stress conditions including cadmium, we generated a SHSY5Y cell cellular model expressing APE1 gene as a fusion with a photoconvertible fluorescent protein Dendra2. Brief graphical summary of the cellular model created and the method used is shown in Figure 1.

4 RESULTS

Experiments were performed with two neuronal cell lines; SF767, a human glioblastoma cell model and SHSY5Y as a neuroblastoma model. Neuronal cells were treated with cadmium as an environmental pollutant able to induce oxidative stress and then assayed for change in cellular viability, change in protein expression and localization. Evaluations of APE1 activities involved in neuroprotections were dissected through the use of three specific APE1 functional inhibitors:

- Compound #3: directly binds and inhibits APE1 DNA repair activity;
- Methoxyamine (MX): indirect inhibitor of APE1 DNA repair activity;



(Tell G et al., BioTechniques, 2013)

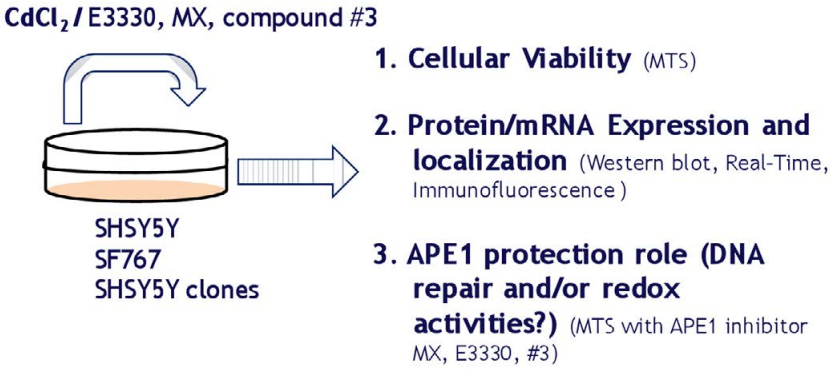


Figure 1: Schematic presentation of SHSY5Y cell lines clones in which endogenous APE1 protein expression can be inducibly knocked-down through RNA interference system by the addition of doxycycline to the medium. To better characterize APE1 trafficking, an APE1 gene siRNA resistant has been cloned in the pDendra vector. Before photoconversion, the chromophore of Dendra2 exists in equilibrium between the neutral state (non-fluorescent) and anionic state (green fluorescence). The excitation of the neutral chromophore to 400nm results in an extremely efficient photoconversion from green to red.

- E3330: inhibitor of APE1 redox function.

Preliminary results showed that cadmium had a great impact on neuronal cell viability impacting on BER enzymes expression. Specifically, both APE1 functions (i.e. DNA-repair and redox function) are crucial for neuronal survival.

5 POTENTIAL NEW PRODUCTS & SERVICES

These studies will shed light on the molecular and functional mechanisms responsible for neurotoxicity and cellular stress due to environmental pollutants. The study of cadmium effect on neuronal toxicity will be of great impact for future researches aimed at exploring the importance of heavy metal-induced oxidative stress as possible etiological factor in the pathogenesis of several neurological disorders. Furthermore, the understanding of the molecular mechanisms regulating APE1 neuroprotective functions will be essential for the development of new therapeutic strategies for prevention/reduction of cadmium induced neurodegeneration.

The neuronal cell lines developed will be of great interest for addressing these issues and represent completely new products for the scientific community that will be possibly patented.

6 CURRENT COLLABORATIONS

6.1 With other researchers

Dr. Sara Tuniz, amministrative manager University of Udine, Prof. Elsa Fabbretti (PP1) University of Nova Gorica, Prof. Stefano Gustincic (LP) SISSA, Trieste, Italy.

7 CONTACT OR COLLABORATIONS NEEDED

Dr. Anton Simeonov, NCGC, NIH, Bethesda MD, USA: Development of small molecules as APE1 functional modulators of its enzymatic activity to protect neuronal cells from oxidative stress and environmental pollutants (i.e. heavy metals).

8 COMMUNICATION TOOLS

Scientific publications granted by the MINA project:

Antoniali G et al., SIRT1 gene expression upon genotoxic damage is regulated by APE1 through nCaRE-promoter elements. Mol. Biol Cell. 2014.

Mantha AK et al., A short review on the implications of base excision repair pathway for neurons: Relevance to neurodegenerative diseases. Mitochondrion. 2013.

Tell G et al., Combining RNAi and in vivo confocal microscopy analysis of the photoconvertible fluorescent protein Dendra2 to study a DNA repair protein. Biotechniques. 2013.

Websites:

http://www.minaproject.eu

http://www.neidos.it/index.pl?pos=03.01&ids=115

https://www.researchgate.net/profile/Gianluca_Tell

9 FUNDS NEEDED

9.1 For basic research (investigation of biological mechanisms): 76.000 €

9.2 For applied research (solutions for real-world problems): 26.000 €

9.2 For pilot & demonstrator activities (to develop a prototype): 4.000 €

10 CONCLUSION

Cadmium is extremely toxic and harmful pollutant leading to neuronal cells death;
Preliminary results demonstrated that depletion of APE1 resulted in a reduction in cell viability after exposure to cadmium confirming the potential role of APE1 in neuroprotection;
Inhibition of both DNA repair and redox activities of APE1 with small molecules sensitizes cell to cadmium treatment supporting the notion that both APE1 functions are essential for neuronal survival;
Cadmium triggers an impairment of the BER pathway determining a reduced expression of BER enzymes which can explain not only its neurotoxic effects but also the potential carcinogenicity of this heavy metal.
The effect of cadmium on furher enzymes of BER is currently under evaluation.

ACKNOWLEDGEMENT

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