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Molecular Regulation and Analysis of Neural Stem and Cancer Cell Characteristics

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ABSTRACT

Every single cell within an organism is constantly facing the demanding task of preserving the integrity of its genomic material to secure proper function and clearance from disease. To accomplish this cells rely on DNA repair mechanisms that are well-conserved throughout evolution and highly specialized in removing particular types of DNA damage. The ability of rapidly repairing DNA lesions is paramount for cells in both the developing and adult central nervous system (CNS), not only to preserve their differentiation potential but also to ensure that post mitotic cells, which comprise the CNS vast majority, are spared and continue to contribute to the maintenance of CNS homeostasis. Due to their putative enhanced repair capacity, neural stem cells (NSCs) may not only play a critical role in sustaining the pool of neural progenitors over the course of neurogenesis, but also in certain conditions, replenish the loss of terminally differentiated cells due to a variety of damage inducing events.

The work presented in this thesis aimed to further explore the significance of DNA glycosylase activity both in neural stem and cancer cells and to develop a probe for rapid assessment of neuronal characteristics in neurons differentiated from NSCs.

In paper I we propose the DNA glycosylases OGG1 and NEIL3 play roles beyond direct removal of oxidized bases and that they are necessary for typical expression levels of genes conferring normal neural stem cell characteristics and multipotency required for differentiation into the various cell lineages found in the mammalian CNS. Further, we show that NEIL3 deficiency can enhance formation of senescence related heterochromatin foci, suggesting an influence in regulatory functions of this cellular pathway.

In paper II we first demonstrate we can generate C6 glioma cells with stem cell-like characteristics (C6SCs), as it could be assessed by an increased ability of these cells to differentiate into astrocytes and neurons after CNTF and VPA treatment respectively. In addition, we show that RNA knockdown of the DNA glycosylase OGG1 in C6SCs affects differentiation potential and increases the histone modification mark, acetylation of histone 3 in lysine 56 (H3K56ac) associated with increased DNA damage response (DDR). This enhancement of the DDR may confer a certain degree of resistance to cancer therapies that should be carefully taken in account.

Finally, in paper III we introduce a new method for the use of a commercially available voltage sensitive dye (VSD), JPW3027, for accurate characterization of neurons differentiated from NCSs, regarding their ability to generate action potentials (AP). We found that extracellular application of this dye improves labeling of cellular processes, and upon excitation it reports changes in fluorescent translating precise AP kinetics, which are of overall superior quality compared to calcium indicators. Furthermore, JPW3027 proved to possess a lesser degree of toxicity, which represents a great advantage when monitoring cells for extended periods of time after dye loading. Finally, we propose the use of a finite element model of the NSC culture cover slip to optimize electrode positioning relatively to the patched cells this way producing an electrical stimulation that is homogenous to all cells. With this approach we are able to predict isopotential fields where electrodes can be placed minimizing the perturbation of cells away from the field of view.