Histochem Cell Biol (2017) 148:473–475
DOI 10.1007/s00418-017-1609-x

EDITORIAL

In focus in HCB

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Accepted: 18 September 2017 / Published online: 21 September 2017

Assessing multiple DNA denaturation pretreatments for BrdU immunostaining

The proliferation state of cells can be assessed via multiple immunostaining protocols, including using antibodies against proliferating cell nuclear antigen (PCNA), Ki-67, and bromodeoxyuridine (BrdU) (Eminaga et al. 2016). Unlike the direct immunostaining for PCNA or Ki-67 on any paraffin-embedded tissue section, BrdU immunostaining requires the prior injection of the thymine analog BrdU into living animals where it incorporates into replicating DNA. The immunostaining procedure then requires section pretreatment with a DNA-denaturing agent to yield single-stranded DNA, a requirement for binding of the anti-BrdU antibody. Typically, denaturation is achieved by section treatment with hydrochloric acid, which may result in a deterioration in both tissue morphological appearance and antigenicity. Since other DNA denaturation agents besides hydrochloric acid have been used in the BrdU immunostaining procedure in previous studies, Molina et al. (2017) have now undertaken a comparative evaluation of the use of a variety of DNA denaturation agents on subsequent BrdU immunostaining, using the transient proliferative rat cerebellar external granular layer (EGL) as a model structure. They found that section pretreatment for partial DNA denaturation with differing concentrations of DNase I, sodium citrate buffer, endonuclease Eco R1, exonuclease III, or hydrochloric acid all resulted in good nuclear immunostaining with the BrdU antibody and acceptable tissue morphology. However, when comparing the effect of the various denaturation agents on cell cycle parameters and phase durations of EGL cells they noted differences in the detection of BrdU-stained cells amongst the reagents, indicating that histological procedures may affect the reproducibility of quantitative analysis of proliferating cells. Taking all of their data into consideration, they recommend that DNase I and citrate buffer may offer less harsh alternatives to hydrochloric acid for DNA denaturation in the BrdU staining protocol.

Autophagy and cell survival in cancer, normal, and embryonic cells

Autophagy is a cellular process by which cytoplasmic components are incorporated into autophagocytic vesicles, which eventually fuse with lysosomes resulting in degradation of cellular constituents (Parzych and Klionsky 2014). Over the years, the understanding of this fundamental cellular process has evolved to delineate its critical role in both physiological and pathophysiological mechanisms. Indeed, autophagy has been shown to play the most interesting role in cancer cells, acting as either a tumor suppressor or tumor promoter, with the response related to the type of tumor cell studied. Moreover, similar to apoptosis, autophagy has also been shown to be an important mechanism during vertebrate development (Aburto et al. 2012). Given these diverse roles of autophagy in cellular constituent degradation processes, Rankov et al. (2017) have performed an in-depth analysis to investigate the effect autophagy has on cell survival in model systems (human cancer cells, human normal cells, and zebrafish embryonic cells) treated with anticancer

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drugs providing a pro-apoptotic environment. For these studies, autophagy was induced with AZD2014 or tamoxifen treatment, whereas it was inhibited by pretreatment of cells with wortmannin or chloroquine. They found the following results: (1) induction of autophagy resulted in a diminished effect of chemotherapeutics on cell lines, whereas inhibition of autophagy resulted in an enhanced cellular effect of the anticancer agents; (2) enhancement of autophagy resulted in a decrease in cellular apoptosis, while inhibition of autophagy produced an increase in apoptosis; and (3) induction of autophagy produced a diminished apoptotic response from chemotherapeutic agents in the tail region of zebrafish embryos, whereas autophagy inhibition yielded increased anticancer drug-induced apoptosis in the tail and retinal regions (see cover image). The authors emphasize the importance of testing the autophagy inducing or inhibition activity of new potential anticancer drugs to aid in predicting the fate of various cell types to chemotherapeutic intervention.

Telomere homeostasis is essential for brain development in the mouse

Telomeric repeat binding factor 2 (TERF2) is a component of the Shelterin complex which covers telomere ends to prevent the induction of the DNA damage response (DDR) (de Lange 2005). Inactivation of TERF2 results in exposed telomere ends which triggers ataxia telangiectasia mutated (ATM)-dependent DDR with subsequent processing of the telomere ends by DNA ligase IV (lig4) (Karlseder et al. 1999). Kim et al. (2017) have investigated whether *Terf2* inactivation induces neuro-specific DDR associated with *Atm* or *Atr* signaling pathways during neurogenesis. Mutant mice with nervous system-specific $Terf2^{Emx1\text{-}Cre}$ plus *nestin* ($Terf2^{Nes\text{-}Cre}$) or homeobox protein *Emx1* ($Terf2^{Emx1\text{-}Cre}$) inactivation were born with greatly abnormal brains. When analyzed at E13.5, $Terf2^{Nes\text{-}Cre}$ embryos showed massive apoptosis throughout the brain, and $Terf2^{Emx1\text{-}Cre}$ embryos displayed apoptosis in the forebrain remnant. At E15.5, a proper brain structure was absent in $Terf2^{Nes\text{-}Cre}$ embryos and the EMX1-expressing brain regions were missing in $Terf2^{Emx1\text{-}Cre}$ embryos. The neural apoptosis during neurogenesis resulting from *Terf2* inactivation could be dramatically reduced by additional *Atm* and *Trp53* inactivation, but not by *Atr* inactivation. Additional inactivation of *Lig4* only moderately corrected the defects in $Terf2^{Emx1\text{-}Cre}$ mice. Together, these results demonstrate the importance of TERF2 to maintain telomere homeostasis in developing mouse brain and that *Terf2* inactivation induces ATM-Trp53 signaling resulting in neural apoptosis and gross brain abnormalities.

Titin-based stretch-sensing proteins are increased after submaximal exhaustive exercise

Titin is a multifunctional skeletal muscle scaffold protein that spans a half sarcomere from the Z-disc to the M-line, and as a mechanosensor senses alterations of mechanical load (Krüger and Kötter 2016). Through its interaction with various proteins, titin is linked to diverse signaling pathways including hypertrophic signaling (Knöll et al. 2011). Koskinen et al. (2017) have investigated the early responses of stress-sensing proteins along titin after fatiguing exercise (exhausting stretch-shortening cycle exercise on a sledge apparatus by continuous drop jumping) on the physical fitness level of individual subjects. mRNA levels for muscle LIM protein (MLP), ankyrin repeat protein Ankrd1/CARP, and muscle-specific RING finger proteins in biopsies from exercised vastus lateralis muscle were significantly increased 3 h after the exercise, and the magnitude of MLP and Ankrd2 responses was related to the proportion of type 1 myofibers. Muscle damage was indicated by the appearance of clusters of HSP27 immunostaining, decreased maximal jump height and increased serum creatine kinase. The authors propose that both degenerative and regenerative pathways are activated very early after, or probably already during the exercise and may represent an initial step of foward adaptive remodeling of the exercised muscle and may also indicate the initiation of myofiber repair.

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