Good report on an investigation of the expression of pcna in granulosa cells of g...

Parts of the World, European Union

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Introduction

In the mouse and other mammalian species, the pool of primordial follicles becomes established during fetal or neonatal life (Oktay et al., 1995). Over the mammal's lifespan, the follicles undergo growth and development, and eventually the initial reserve of follicles begins to diminish during the mammal's midlife. Briefly, follicular cells move from a primordial phase, in which little DNA synthesis is taking place, to enter a primary and then secondary stage, in which the granulose cells undergo intense proliferation and high levels of DNA synthesis can be observed. Antral and post- ovulation stages follow (Oktay et al., 1995). Folliculogenesis can be tracked using immunochemistry techniques and a select group of protein markers which appear at various stages during follicular development. One approach used is to follow a protein that is highly expressed during follicular growth. Another would be to track a protein which is present at specific morphological stages of development (Ino and Chilba, 2000).

Proliferating cell nuclear antigen (PCNA) and zona pellucid C (ZPC) are

proteins that are involved in the mammalian fertilization process. PCNA is a relatively small protein (36 kDal in size) that is highly conserved across both animal and plant species (Fortune et al., 2000). PCNA is expressed during the early G and S phases of the cell cycle and acts by tethering DNA to the polymerase during replication. It is for this reason that PCNA is detected in tissues at sites within the cell where DNA synthesis is actively taking place (Fortune et al., 2000). ZP protein C, on the other hand, is a sulfated glycoprotein that polymerizes along with other ZP proteins into filaments that surround the oocyte. The zona pellucida (ZP) is the extracellular matrix which mammalian sperm first encounter during fertilization and is generated when oocytes begin to grow (Wassarman and Litscher, 2008).

In the following report, an immunohistochemistry assay was performed to determine PCNA and ZPC distribution in tissue samples taken from mouse ovaries. This was followed by a Western blot analysis of SDS protein extracts from mouse liver, brain, spleen and ovary tissues. The Western blot was used to test for PCNA expression in these tissues. The report concludes with a discussion as to whether PCNA and ZPC are demonstrated to be useful protein markers for follicular development.

Materials and Methods

Please refer to the lab manual.

Results:

Immunoblotting:

Figure 1. Immunoblot showing PCNA expression from different tissue simple

Lane 1-protein marker, Lane 2-Brain extract, Lane 3-Liver extract, Lane 4-Ovary extract, Lane 5-Spleen extract

Immunohistochemistry Analysis:

(a) (b)

Figure 2. PCNA expression immunohistochemistry in Bouins fixed secondary follicles (a) PCNA (b) control.

- (b)

Figure 3. PCNA expression in immunohistochemistry. Paraformaldehyde fixed secondary follicle. Note the exclusion from the cell in (a) but not in (b).

(a) (b) (c)

Figure 4. ZPC expression by immunohistochemistry in Bouins fixed specimens (a) primordial follicle (b) primary follicle (c) secondary follicle. Note the increase in the intensity of ZPC staining in the secondary follicle.

- (b)

Figure 5. ZPC expression by immunohistochemistry in Paraformaldehyde fixed specimens (a) Antral follicle (b)Tertiary foliicle.

Discussion

The purpose of this laboratory was to examine techniques utilized for immunchemistry. This involved examining levels of PCNA and ZPC in mouse tissue samples and included both Western blot analysis and immunohistochemistry techniques. The results of this experiment demonstrate that both PCNA and ZPC represent good protein markers of follicular growth and development. Western blot analysis has demonstrated that PCNA is present in all of the tissues examined. It is interesting to note in

Figure 1 that the levels of PCNA were lowest in the extracts taken from the brain tissue, rather than the liver, ovary or spleen tissue extracts (compare lane 2 with lanes 3-5). This is because there is less DNA synthesis taking place in brain tissue in general, since brain cells in general are not actively undergoing division. It is possible that the PCNA found to be expressed in this tissue may play a role in a DNA repair mechanism that takes place in postmitotic nonproliferating cell nuclei. Neurons are cells which are nondividing and have long lifespans, and thus could be subject to genetic mutations over time, and thus require DNA repair. Cells are actively dividing in liver, spleen and ovary tissues, which is why PCNA levels are highest in these tissues (Ino and Chiba, 2000). It could be argued that the same amount of PCNA is being produced in these three tissues, as the banding intensity is uniform. A more quantitative analysis would be needed to compare PCNA levels in each of these tissues.

Based on the Western blot analysis, two bands can be observed. The lower band most likely represents a monomer of PCNA, at a molecular weight of 36 kDa. The larger band, of molecular weight ~100 kDa, could represent the trimeric ring-like structure that PCNA assembles into in order to clamp onto DNA (Bowman et al., 2004, Moldovan et al., 2007).

Based on the immunohistochemistry results, the relative distribution of PCNA and ZPC could be identified in mouse ovary tissues. In Figure 2a, PCNA could be detected in secondary follicles, but not in the primary antibody control sample. Specifically, staining was present in the columnar granulosa cells of the secondary follicle. PCNA could also be detected in secondary follicles in Figure 3. Immunocytochemical staining for the presence of PCNA within

follicular cells tended to vary depending on the specific stage of follicular growth (Fortune et al., 2000, Johnson et al., 2004). For example, little pCNA could be found in primary follicles, whereas high levels of PCNA were detected in secondary follicles. This is the point where DNA synthesis is actively taking place. Similarly, ZPC expression was observed to be significantly enhanced in the secondary follicles (Figure 4, compare a and b with c). ZPC expression was also high in the antral follicle, rather than the tertiary follicle (Figure 5, compare b and c). The immunocytochemical staining results for ZPC support the results for PCNA, indicating that both proteins can be used as markers to locate sites of high levels of DNA synthesis and cell division during follicular development. These proteins are not present in the primordial follicles, because they are not actively undergoing cell division, nor has the extracellular matrix undergone development.

The results of this laboratory indicate that both PCNA and ZPC are adequate markers to examine follicular development in mouse ovary. The laboratory also served as a means to learn the techniques of immunoblot & immunohistochemical analysis.

References

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