In focus in HCB

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Zw10 is essential for proper meiotic maturation of mouse oocytes

Faithful chromosome segregation during mitosis is ensured by correct spindle microtubule attachment to kinetochores located within the centromeric heterochromatin region of chromosomes (Cheeseman and Desai 2008; Luckner and Wanner 2018). The kinetochore–microtubule attachment during metaphase is controlled by the multiprotein spindle assembly checkpoint complex (Gardner and Burke 2000). Zw10 is a protein of the highly conserved RZZ (Rod/Zwilch/Zw10) complex of the spindle assembly checkpoint and its mutations result in chromosome mis-segregation and high rates of aneuploidy in somatic cells (Scaerou et al. 2001). Here, Park et al. (2019) report the essential function of Zw10 during mouse oocyte meiosis. By immunofluorescence, Zw10 was observed throughout the cytoplasm and nucleus during the germinal vesicle stage, which dramatically changed shortly after the germinal vesicle breakdown to its positioning at the kinetochores and spindle pole where it remained during metaphase I and II (see cover image). Double immunofluorescence using the anticentromere antibody confirmed the kinetochore association of Zw10 in metaphase I stage oocytes. Following Zw10 siRNA knockdown, premature polar body extrusion occurred, which was due to failure to recruit the spindle assembly checkpoint protein

Mad2 to kinetochores. The Zw10 knockdown caused chromosome misalignment by impaired kinetochore-microtubule attachment and resulted in aneuploidy. Since Zw10 levels decreased with maternal age, it was proposed that this is associated with the age-related increase in aneuploidy. Together, this work on mouse oocytes establishes the association of Zw10 with kinetochores and its function as an essential spindle assembly checkpoint protein.

Reduced fat may not always be a good thing: desmosome dynamics and plasma membrane cholesterol content

Desmosomes, as components of intercellular adhering junctions, are intimately involved in providing strength and integrity for epithelial cellular adhesion (Waschke 2008). The molecular components of desmosomes, cadherins of the desmoglein and desmocollin subtypes, together with a cytoplasmic plaque domain are well studied and known (Nekrasova and Green 2013). Less well known is how intraplasma membrane components, such as lipids serve to aid in the assembly and regulation of desmosome dynamics. In an earlier investigation, Resnik et al. (2011) demonstrated that desmosome assembly required membrane cholesterol and lipid modifications. They have now extended this prior work to investigate how cellular cholesterol content affects the lateral mobility and distribution of the desmosomal protein desmocollin 2, as well as the overall adhesive strength of desmosomes in an MDCK cell model (Resnik et al. 2019). Cells were transfected with a fluorescent desmocollin 2a chimera (Dsc2-YFP), and following cholesterol depletion from the plasma membrane, they were imaged by confocal scanning laser microscopy and transmission electron microscopy. Moreover, in-depth quantitative analyses were performed at both light microscopic and electron microscopic resolution by performing (1) fluorescence recovery after photobleaching (FRAP; to determine lateral mobility of Dsc2-YFP in the membrane); (2) re-scanning confocal microscopy with

Two Original Papers, one describing the role of a specific protein in regulating mouse oocyte meiosis, and the other the effect of plasma membrane cholesterol content on desmosome dynamics and adhesion strength, as well as one Short Communication providing details on the effect of tissue storage in *RNA Later* on subsequent histology and immunohistochemistry highlight our overview of the September issue of HCB.

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fluorescence intensity measurements; and (3) immunoelectron microscopic detection of the membrane lipid osteolyisin/pleurotolysin B (OlyA/PlyB) with both pre-embedding and ultrathin cryosections. By chemically depleting cholesterol predominantly from the plasma membrane, and using the multiple imaging techniques just described, the authors found that depletion of cellular cholesterol resulted in a decrease in the lateral mobility and dispersion of desmocollin 2, leading to an overall decrease in adhesion strength and ultrastructural characteristics of the desmosomes. Their immunoelectron microscopy results showing the localization of OlyA/PlyB at the plasma membrane are also the first to demonstrate the co-association of desmosomes with cholesterol-enriched regions of the plasma membrane. Overall, this excellent study showed that desmosomes are stabilized not only through protein-protein interactions, but that plasma membrane cholesterol is involved in regulating the dynamic properties of these intercellular junctions.

Tissue storage prior to formalin fixation: better “RNA”Later than never?

The search for the ideal fixative for optimal tissue morphological preservation combined with molecular antigen preservation has been ongoing for over 75 years since the introduction of the first immunohistochemical technique (Coons et al. 1942). For tissues embedded in paraffin wax, such as the majority of specimens processed for diagnostic pathology, formalin fixation has been the method of choice since the late nineteenth century. Although resulting in overall excellent morphological preservation at the light microscopic level, formaldehyde unfortunately often leads to detrimental effects on tissue antigens (Puchtler and Meloan 1985). Moreover, in some instances, immediate fixation of tissue in formalin may not be practical. In this case, other tissue preservatives such as RNALater, which was introduced as a storage reagent for preserving the tissue integrity of RNA, have been assessed for their utility in preserving tissue morphology and antigenicity (Wang et al. 2018). Suhovskih et al. (2019) have now followed-up on such studies to investigate the use of RNALater for storing unfixed mouse tissues for 7 or 14 days prior to subsequent formalin fixation, followed by assessment of tissue morphology and antigen preservation by two pathologists. Their results indicated a tissue-specific effect of storage in RNALater on both morphology and antigen preservation. Interestingly, only lung tissue morphology appeared somewhat unaltered by storage in RNALater, whereas brain, liver, and kidney all showed various artifactual effects. Moreover, immunoreactivity for

β -actin, glial fibrillary acidic protein, and glycosaminoglycan chondroitin sulfate were all variously affected by storage for both 7 and 14 days in RNALater. These results warrant the use of caution for both routine histological analysis and immunohistochemistry when tissues have been stored in RNALater prior to conventional chemical fixation. Clearly, more detailed studies are required for tissues and antibodies for specific applications.

References

- Cheeseman IM, Desai A (2008) Molecular architecture of the kinetochore–microtubule interface. *Nat Rev Mol Cell Biol* 9(1):33–46. <https://doi.org/10.1038/nrm2310>
- Coons AH, Creech HJ, Jones RN, Berliner E (1942) The demonstration of pneumococcal antigen in tissues by the use of fluorescent antibody. *J Immunol* 45:159–170
- Gardner RD, Burke DJ (2000) The spindle checkpoint: two transitions, two pathways. *Trends Cell Biol* 10(4):154–158
- Luckner M, Wanner G (2018) Precise and economic FIB/SEM for CLEM: with 2 nm voxels through mitosis. *Histochem Cell Biol* 150:149–170. <https://doi.org/10.1007/s00418-018-1681-x>
- Nekrasova O, Green KJ (2013) Desmosome assembly and dynamics. *Trends Cell Biol* 23:537–546. <https://doi.org/10.1016/j.tcb.2013.06.004>
- Park Y, Kim J-S, Oh JS (2019) Zw10 is a spindle assembly checkpoint protein that regulates meiotic maturation in mouse oocytes. *Histochem Cell Biol*. <https://doi.org/10.1007/s00418-019-01800-9>
- Puchtler H, Meloan SN (1985) On the chemistry of formaldehyde fixation and its effects on immunohistochemical reactions. *Histochemistry* 82:201–204
- Resnik N, Sepcic K, Plemenitas A, Windoffer R, Leube R, Veranic P (2011) Desmosome assembly and cell–cell adhesion are membrane raft-dependent processes. *J Biol Chem* 286:1499–1507. <https://doi.org/10.1074/jbc.M110.189464>
- Resnik N, de Luca GMR, Sepcic K, Romih R, Manders E, Veranic P (2019) Depletion of the cellular cholesterol content reduces the dynamics of desmosomal cadherins and interferes with desmosomal strength. *Histochem Cell Biol*. <https://doi.org/10.1007/s00418-019-01797-1>
- Scaerou F, Starr DA, Piano F, Papoulas O, Karess RE, Goldberg ML (2001) The ZW10 and rough deal checkpoint proteins function together in a large, evolutionarily conserved complex targeted to the kinetochore. *J Cell Sci* 114(Pt 17):3103–3114
- Suhovskih AV, Kazanskaya GM, Volkov AM, Tsidulko AY, Aidagulova SV, Grigorieva EV (2019) Suitability of RNALater solution as a tissue-preserving reagent for immunohistochemical analysis. *Histochem Cell Biol*. <https://doi.org/10.1007/s00418-019-01799-z>
- Wang M, Ji X, Wang B, Li Q, Zhou J (2018) Simultaneous evaluation of the preservative effect of RNALater on different tissues by biomolecular and histological analysis. *Biopreserv Biobank* 16:426–433. <https://doi.org/10.1089/bio.2018.0055>
- Waschke J (2008) The desmosome and pemphigus. *Histochem Cell Biol* 130:21–54. <https://doi.org/10.1007/s00418-008-0420-0>

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