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EDITORIAL

In focus in HCB

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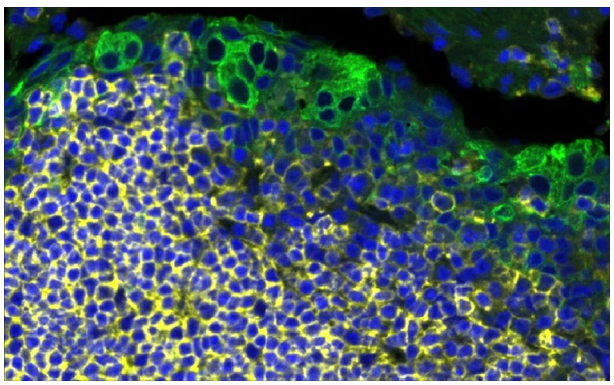

In this month's editorial, we will highlight three manuscripts describing (1) the selective targeting and endocytosis of three different plant lectins in human bladder cancer cell lines and patient-derived uroepithelial tumor tissue as possible vectors to deliver therapeutic agents; (2) changes in smooth muscle layers and myenteric plexus in the small bowel as adaptations to short bowel syndrome; and (3) the feasibility of using paraffin-embedded human biopsy samples initially subjected to synchrotron-based x-ray phase-contrast imaging for subsequent standard histological and histochemical evaluation. Also, we hope you take note of and peruse the accompanying Editorial in this issue which introduces the implementation by *Histochemistry and Cell Biology* of new guidelines for the use of Large Language Models (including ChatGPT).

Lectins for possible therapeutical targeting of urinary bladder carcinoma

Urinary bladder carcinoma can be treated by surgery combined with intravesicular instillation of mitomycin C or Bacillus Calmette–Guérin. Furthermore, lectins have been experimentally used for targeted drug delivery to urothelial tumors, since glycans of cell surface glycoproteins undergo compositional changes during bladder cancer development and progression (Neutsch et al. 2014; Plattner et al. 2008; Zupancic et al. 2014). Moreover, progression of bladder carcinoma to high-grade, invasive tumors has been shown to be associated with increased endocytic activity (Kreft et al. 2009; Lojk et al. 2018). In continuation of previous work in rats and mice (Zupancic et al. 2014), Resnik et al. (2023) performed lectin binding and endocytosis analyses in vitro using human bladder carcinoma cells and ex vivo on biopsy specimens. They employed fluorescent and gold-labeled Jacalin, *Datura stramonium*, and *Amaranthus caudatus* lectins to study (1) normal porcine urothelial cells, the low-grade human urothelial papilloma cell line RT4, and the high-grade urothelial cancer cell line T24, and (2) biopsy samples from normal human bladder, papilloma, noninvasive low-grade urothelial carcinoma, invasive low-grade urothelial carcinoma with invasion into the lamina propria, invasive high-grade urothelial carcinoma with invasion into the lamina propria, and invasive high-grade urothelial carcinoma with invasion into the muscularis propria. The lectin studies were complemented by immunofluorescence and immunogold labeling, as well as western blotting for uroplakin. For endocytosis experiments, fluorescein isothiocyanate (FITC)-conjugated lectins were combined with tetramethylrhodamine (TRITC)-labeled 3 kDa or 70 kDa dextran. Without particularizing the results of their highly detailed studies, the authors were able to show that discrimination between normal and cancer urothelial cells and between low- and high-grade cancer cells is possible by lectin binding, and that, as they had previously shown, macropinocytosis is the main endocytic pathway of the studied lectins in urothelial

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cancer cells. They propose "that lectins should be used as targeting ligands for innovative drug delivery systems because of their enhanced uptake into cancer urothelial cells through macropinocytosis."

Muscles and nerves in short bowel syndrome

Short bowel syndrome (SBS) is a malabsorptive condition typically resulting from massive surgical bowel resection of the small intestine, with the remaining portion of the gastrointestinal tract unable to functionally compensate for the missing section, resulting in malabsorption and maldigestion (Massironi et al. 2020). Since the remaining portions of the small intestine often exhibit dilatation, Khasanov et al. (2023) have now investigated whether this pathological response could be the result of alterations in smooth muscle layers and the gut enteric nervous system (ENS). To address this question, they performed quantitative immunohistochemistry, cytochemistry, and morphometric analyses on paraffin sections of segments of jejunum and ileum from experimental rats and human samples (the choice of jejunum and ileum segments allowed comparisons of bowel regions located orally and anally from the anastomosis). Sections were stained with antibodies against smooth muscle actin (to determine smooth muscle cell layers), nestin (a marker for neuronal plasticity), PGP9.5 (neuronal marker for human cells), and picrosirius red to delineate collagen fibers. Images were acquired by both transmitted light and fluorescence microscopy, and subjected to various morphometric analyses. Their comprehensive results can be divided into those obtained with the rat tissues and those with the human samples. For the rat tissues, they found that, compared to sham-treated animals, animals subjected to short bowel resection displayed (1) increased thickness of both longitudinal and circular smooth muscle cell layers; (2) increased diameter of both sections from the small bowel; (3) increased muscle circumference due to increases in the numbers of smooth muscle cells; (4) increased amount of collagen in the area of the muscle circumference; (5) increased size of nuclei (measured by nuclear area), but decreased number of nuclei in a given area, indicating hypertrophy of the nuclei and muscle cells; (6) increased number of nestin-positive myenteric ganglia; (7) an increase in the total area of the myenteric ganglia; (8) higher ratio of samples with a greater amount of nestin-positive areas in the muscle layers; and (9) correlation of nestin positivity with muscle cell hypertrophy. They then sought to determine whether the accumulated rat experimental data correlated with tissue presentation in the human samples. Their results with the human samples showed (1) a significant increase in the number of neural stem cells in samples from SBS patients compared to control samples; (2) no difference in the proportion of neuronal cells in the myenteric plexus in SBS samples as compared to control samples; and (3) an increase in the ratio of the proportion of stem cells to neurons in the myenteric plexus in samples from SBS patients compared to those from control samples. Overall, their results indicated that SBS is accompanied by smooth muscle hypertrophy, proliferation of collagen fibers in the smooth muscle layers, and ENS neuroplasticity. Moreover, the authors conclude that the intestinal ENS represented by the myenteric plexus is intimately connected to alterations in intestinal smooth muscle layers, resulting in intestinal adaptations to SBS.

All is feasible after FFPE

Formalin fixation followed by paraffin embedding (FFPE) not only is the gold standard for diagnostic histology performed on tissue sections, but also permits analytical immunohistochemistry and molecular analyses of extracted nucleic acids. Recent technical developments such as light sheet microscopy of optically cleared tissues (Almagro et al. 2021; Glaser et al. 2017), micro-computed tomography (Teplov et al. 2019), and X-ray phase-contrast imaging (X-PCI) (Borisova et al. 2021; Dejea et al. 2019; Walsh et al. 2021) have offered new possibilities for the 3D analysis of FFPE tissues by virtual slicing of the tissue blocks. Notably, X-PCI when combined with synchrotron offers high contrast for soft tissues (Grandl et al. 2013). However, X-ray irradiation of FFPE blocks may be harmful for subsequent analysis of molecular components in sections prepared from irradiated blocks. In their present investigation, Li and coworkers (Li et al. 2023) performed histological, immunohistochemical, and molecular analyses of sections prepared from FFPE blocks that were previously subjected to synchrotron-based X-PCI. They analyzed surgical specimens of human liver, lung, and kidney, and calculated the cumulative radiation dose, as well as the dose per scan at different magnifications (1× –5.8 µm/voxel, 4× –1.625 µm/voxel and 10× –0.65 µm/voxel). Afterwards, tissue sections were stained by hematoxylin and eosin (H&E) and periodic acid–Schiff (PAS), immunohistochemistry was performed for PAX 8, CK7, CD10, and TFF1, genomic DNA integrity was determined, and EGFR mutational analysis was performed by droplet digital PCR. The authors demonstrated that synchrotron-based X-PCI is compatible with routine histochemistry and immunohistochemistry, as already shown for related techniques (Albers et al. 2021; Norvik et al. 2020; Pinkert-Leetsch et al. 2023; Saccomano et al. 2018). Their new data from DNA and RNA analyses indicate that synchrotron-based X-PCI does not impede DNA integrity, nor does it affect RNA sequence analysis. Of note, these results were obtained over a kGy range of radiation doses. Appropriately,

the authors conclude that it is feasible to perform X-PCI on blocks of FFPE human biopsies without impairing subsequent diverse analysis of sections.

References

Albers J, Svetlove A, Alves J et al (2021) Elastic transformation of histological slices allows precise co-registration with microCT data sets for a refined virtual histology approach. Sci Rep 11:10846. https://doi.org/10.1038/s41598-021-89841-w

Almagro J, Messal HA, Zaw Thin M et al (2021) Tissue clearing to examine tumour complexity in three dimensions. Nat Rev Cancer 21:718–730. https://doi.org/10.1038/s41568-021-00382-w

Borisova E, Lovric G, Miettinen A et al (2021) Micrometer-resolution X-ray tomographic full-volume reconstruction of an intact post-mortem juvenile rat lung. Histochem Cell Biol 155:215–226. https://doi.org/10.1007/s00418-020-01868-8

Dejea H, Garcia-Canadilla P, Cook AC et al (2019) Comprehensive analysis of animal models of cardiovascular disease using multi-scale X-ray phase contrast tomography. Sci Rep 9:6996. https://doi.org/10.1038/s41598-019-43407-z

Glaser AK, Reder NP, Chen Y et al (2017) Light-sheet microscopy for slide-free non-destructive pathology of large clinical specimens. Nat Biomed Eng 1:0084. https://doi.org/10.1038/s41551-017-0084

Grandl S, Willner M, Herzen J et al (2013) Evaluation of phase-contrast CT of breast tissue at conventional X-ray sources—presentation of selected findings. Z Med Phys 23:212–221. https://doi.org/10.1016/j.zemedi.2013.02.005

Khasanov R, Svoboda D, Tapia-Laliena MA et al (2023) Muscle hypertrophy and neuroplasticity in the small bowel in short bowel syndrome. Histochem Cell Biol. https://doi.org/10.1007/s00418-023-02214-4

Kreft ME, Romih R, Kreft M, Jezernik K (2009) Endocytotic activity of bladder superficial urothelial cells is inversely related to their differentiation stage. Differentiation 77(1):48–59. https://doi.org/10.1016/j.diff.2008.09.011

Li K, Dejea H, De Winne K et al (2023) Feasibility and safety of synchrotron-based X-ray phase contrast imaging as a technique complementary to histopathology analysis. Histochem Cell Biol. https://doi.org/10.1007/s00418-023-02220-6

Lojk J, Bregar VB, Strojan K, Hudoklin S, Veranic P, Pavlin M, Kreft ME (2018) Increased endocytosis of magnetic nanoparticles into cancerous urothelial cells versus normal urothelial cells. Histochem Cell Biol 149(1):45–59. https://doi.org/10.1007/s00418-017-1605-1

Massironi S, Cavalcoli F, Rausa E et al (2020) Understanding short bowel syndrome: current status and future perspectives. Dig Liver Dis 52(3):253–261. https://doi.org/10.1016/j.dld.2019.11.013

Neutsch L, Eggenreich B, Herwig E, Marchetti-Deschmann M, Allmaier G, Gabor F, Wirth M (2014) Biomimetic delivery strategies at the urothelium: targeted cytoinvasion in bladder cancer cells via lectin bioconjugates. Pharm Res 31(3):819–832. https://doi.org/10.1007/s11095-013-1204-3

Norvik C, Westöö CK, Peruzzi N et al (2020) Synchrotron-based phase-contrast micro-CT as a tool for understanding pulmonary vascular pathobiology and the 3-D microanatomy of alveolar capillary dysplasia. Am J Physiol Lung Cell Mol Physiol 318:L65–L75. https://doi.org/10.1152/ajplung.00103.2019

Pinkert-Leetsch D, Frohn J, Ströbel P et al (2023) Three-dimensional analysis of human pancreatic cancer specimens by phase-contrast based X-ray tomography—the next dimension of diagnosis. Cancer Imaging 23:43. https://doi.org/10.1186/s40644-023-00559-6

Plattner VE, Wagner M, Ratzinger G, Gabor F, Wirth M (2008) Targeted drug delivery: binding and uptake of plant lectins using human 5637 bladder cancer cells. Eur J Pharm Biopharm 70(2):572–576. https://doi.org/10.1016/j.ejpb.2008.06.004

Resnik N, Višnjar T, Smrkolj T et al (2023) Selective targeting of lectins and their macropinocytosis in urothelial tumours: translation from in vitro to ex vivo. Histochem Cell Biol. https://doi.org/10.1007/s00418-023-02224-2

Saccomano M, Albers J, Tromba G et al (2018) Synchrotron inline phase contrast μCT enables detailed virtual histology of embedded soft-tissue samples with and without staining. J Synchrotron Rad 25:1153–1161. https://doi.org/10.1107/S1600577518005489

Teplov A, Yagi Y, Tabata K et al (2019) Development of standard operating procedure (SOP) of micro-computed tomography (micro-CT) in pathology. Diagnostic Pathol. https://doi.org/10.17629/WWW.DIAGNOSTICPATHOLOGY.EU-2019-5:273

Walsh CL, Tafforeau P, Wagner WL et al (2021) Imaging intact human organs with local resolution of cellular structures using hierarchical phase-contrast tomography. Nat Meth 18:1532–1541. https://doi.org/10.1038/s41592-021-01317-x

Zupancic D, Kreft ME, Romih R (2014) Selective binding of lectins to normal and neoplastic urothelium in rat and mouse bladder carcinogenesis models. Protoplasma 251(1):49–59. https://doi.org/10.1007/s00709-013-0524-9