In focus in HCB

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Antibodies applied for immunohistochemistry have revolutionized diagnostic pathology. Southwestern histochemistry represents an alternative, not antibody-based method to detect gene-specific transcription regulatory factors by haptenized synthetic double-stranded oligo-DNA probes in tissue sections (Koji 1999; An et al. 2012). In this issue of *Histochem Cell Biol*, Bukari et al. (2017) review various practical aspects of Aptahistochemistry for the histochemical protein localization in sections of formaldehyde-fixed and paraffin-embedded tissues. Aptahistochemistry applies DNA or RNA aptamers that are in vitro-synthesized short, single-stranded oligonucleotides for high affinity and specific binding to the respective protein targets. Aptamers can be substituted at the 3' or 5' end by biotin, horseradish peroxidase or different fluorophores. Furthermore, they are small (approximately 15 kD) as compared to the much larger antibody molecule, which permits better penetration into tissue sections and more effective epitope recognition. Similar to immunohistochemistry, Aptahistochemistry is performed after antigen retrieval by heating or enzyme digestion. Despite resemblances in many technical aspects with immunohistochemistry, Aptahistochemistry provides advantages in terms of costs and versatility.

The G-actin-sequestering protein Thymosin β 4 (T β 4) is involved in a myriad of cellular functions, including cell division, migration, tissue regeneration and healing. Moreover, its role in stem cell proliferation in the embryonic

chicken optic tectum (OT) has also been established (Wirsching et al. 2012). This same group (Lever et al. 2017) has extended their previous investigation by now performing a quantitative evaluation of the effect of overexpression of T β 4 on developing chick OT during early stages of development (E4 and E6). They used an electroporation technique to overexpress T β 4 at E2 in neural stem cells comprising the mesencephalon, representing one specific OT-hemisphere, with the opposite hemisphere serving as a control. Using multifuorescence immunolabeling (see cover image) for mitotic cells, neuronal cells, and glial cells together with spinning disk confocal microscopy, they analyzed quantitatively a number of morphological and cellular parameters, including hemisphere surface, tangential length of ventricle and pia, cell number and cell density. Their careful analysis demonstrated that overexpression of T β 4 resulted in a tangential expansion of the OT hemisphere compared to the non-treated opposite hemisphere, and that the total cellular density increased, and in particular post-mitotic neuronal cells. Since the chick OT has similar features to the mammalian cortex, the authors speculate that T β 4 may likewise be involved in regulating proliferation of neuronal and glial cells in developing mammalian brain.

In another investigation of mechanisms acting during tissue and organ development, Sammar and colleagues (2017) explored the fascinating fetomaternal immune tolerance which occurs in the placenta during pregnancy. Since the signal transduction molecule CD24 using its abundant sialic acid moieties interacts with Siglec-10 (member of the Siglec family which are Ig-like type 1 transmembrane proteins with IgV domains able to bind to sialic acids) to effect immune suppression, Sammar et al. (2017) investigated whether these two molecules may be involved in mediating immune suppression to protect the fetus during early

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pregnancy. Employing a variety of techniques, including ELISA assays, Western blots, flow cytometry, immunohistochemistry and immunofluorescence the authors demonstrated (1) purification of CD24 from placental tissues; (2) binding interactions between CD24 and recombinant Siglec-10; (3) immunohistochemical detection of CD24 in first trimester placental tissues and placental bed (decidua); (4) co-localization of CD24 and Siglec-10 in epithelial cells of uterine glands and first trimester decidual cells. The authors present a schematic model for the proposed CD24-Siglec-10 interaction, which should lead to further investigations testing the model's predictions.

Bohlender et al. (2017) investigated the relationship between the renal tissular renin–angiotensin and bradykinin–kallikrein system and the renal sympathetic innervation in the control of kidney function. As a model, they used the unilateral renal denervation system which results in ipsilateral natriuresis and diuresis and enhanced sympathetic nervous output to the contralateral kidney. Six days after unilateral (left) kidney denervation, renin, angiotensin III, dopamine and norepinephrine concentrations were decreased in the denervated kidneys, whereas renal bradykinin and angiotensin I and II concentrations decreased bilaterally in denervated versus sham-operated rats, and angiotensin III/II increased in the right versus denervated kidney or sham-operated rats. Furthermore, the angiotensinergic and catecholaminergic innervation of the right (not denervated) kidneys was not changed as compared to kidneys of sham-operated rats. Together, this demonstrated that bilateral and side-specific changes of the renal–angiotensin and bradykinin concentrations may play a role in this model.

Human type 1 diabetes mellitus is associated with beta cell autoimmunity, and various forms of beta cell stress in addition have been shown to lead to impairment of beta cell function. Furthermore, excess reactive oxygen species produced by beta cells may mediate beta cell destruction during the very early stages of the disease. Among them, nitric oxide produced by beta cells together with superoxide anion may form peroxynitrite. In turn, peroxynitrite-mediated nitration of beta cell proteins may impair their function. Martin et al. (2017) have performed an immunohistochemical study of nitrotyrosine expression during

progression of type 1 Diabetes mellitus in human patients. In non-diabetic, autoantibody-negative cases, nitrotyrosine expression was observed in up to 58.8% of insulin-positive islets. In recently diagnosed diabetes cases, in five out of six cases up to 85.7% of islets were positive. In long duration cases, 60% (one case) and 100% (two cases) of islets were positive and in three cases with insulin-negative islets, nitrotyrosine was undetectable. This indicates that nitrotyrosine expression is increased in recent-onset and long-standing type 1 diabetes mellitus. Whether beta cell nitration plays a role in the pathogenesis of the diseases remains to be shown.

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