In focus in HCB

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In this month's Editorial, we highlight a review article describing the epigenomic changes occurring during mouse spermatogenesis resulting in the induction of germ cell apoptosis and three original articles providing insights into the effects of the modification of the carbohydrate recognition domain (CRD) of a lectin on binding to its glycoconjugate reaction partners; the expression of the histone deacetylase Sirtuin 1 in uterine cancers and its correlation with clinical parameters, and finally, use of the chorioallantoic membrane (CAM) of the chicken embryo as a model to analyze the growth behavior of three invasive human melanoma cell lines. We hope you enjoy this month's offerings!

Germ cell apoptosis and epigenomes in mouse spermatogenesis

Professor Koji, from Nagasaki University Graduate School of Biomedical Sciences, had been invited to present the “Histochemistry and Cell Biology” Lecture at the 16th International Congress of Histochemistry and Cytochemistry, to be held in Prague this year. As we are all aware, due to the COVID19 pandemic this meeting has been postponed until September of 2021. Professor Koji will deliver his lecture at the meeting next year, but in the meantime, he has provided a review of the topic of his presentation, which we are most pleased to publish in this issue of the journal. In this review article, Koji and Shibata (2020) present a timely and fascinating summary of the epigenomic changes found to occur during mouse spermatogenesis, and thought to result in the induction of germ cell apoptosis. They begin the review by summarizing the events occurring during mouse

spermatogenesis, including the events surrounding germ cell apoptosis in both fetal and adult testes. Next, they detail the general features of epigenomes through epigenetics, which they define as “the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in the DNA sequence” (Russo et al. 1996). In this section, they focus specifically on the epigenetic factors of DNA methylation and histone modifications. They go on to describe (immuno)histochemical methods to investigate DNA methylation in mouse spermatogenesis, including HELMET (histo endonuclease linked detection of methylated DNA), which the authors developed (Koji et al. 2008), and histone modifications. Experiments to assess the *in vivo* manipulation of histones through techniques such as genetic engineering and their effects on germ cell apoptosis are then described in detail. Finally, they discuss the effects of endocrine disruptors such as diethylstilbestrol (DES) and environmental toxins, including bisphenol A (BPA) on epigenetic parameters during spermatogenesis. The last section of the review entitled “Perspective” contains a brief summary of the covered topic, and includes a strong endorsement for the use of a variety of histochemical and immunohistochemical techniques for the continued advancement of research into the linkage between epigenetics in spermatogenesis.

Protein design and (gal)lectin activity

The pairwise interaction between lectins and their glycoconjugate reaction partners in animal tissues is of importance in many patho-physiological processes (Cummings 2019; Duan and Paulson 2020; Kaltner et al. 2019). In their present study, García Caballero et al. (2020) aimed to identify principles of structure–activity relationships that underlie specificity of recognition and the ensuing post-binding processes. To this end, they focused on the architecture of the carbohydrate recognition domain (CRD) of the multifunctional human galectin-3 (Gal-3) and engineered variants with different modular assembly to relate protein design to

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Fig. 1 Schematic illustration of different types of modular display of vertebrate galectins (a) and of the Gal-3 CRD-based variants tested in the study by García Caballero et al. (2020) (b). The set of variants based on the Gal-3 CRD consists of a pair of homodimers and two pairs of heterodimers. From García Caballero et al. (2020)

activity. Specifically, they studied the binding properties of (full-length) wild-type Gal-3 and its CRD, a natural change of structure by proteolysis in situ, and a panel of six homo- and heterodimeric variants (Fig. 1) by isothermal titration calorimetry, on glycan arrays and in tissue sections.

They observed that the presence of the N-terminal tail did not markedly affect the thermodynamics of disaccharide binding, and that free disaccharides and a wide array of glycomolecules placed homogeneously on a chip interacted with this CRD in all tested proteins rather uniformly, that is mostly irrespective of protein design. However, the architecture of the CRD did matter when sections of murine epididymis and jejunum fixed in Bouin's fluid and embedded in paraffin were analyzed, since striking cell type-dependent and tissue-regional differences in staining pattern were observed. Furthermore, the susceptibility to inhibition by a tetravalent glycocluster was markedly different for the wild-type as compared to the homodimeric variant proteins.

Therefore, a main conclusion drawn from the study was “that protein design matters: the type of CRD presentation can have a profound bearing on whether basically suited oligosaccharides, which for example tested positively in an array, will become binding partners “in situ.”

Expression of Sirtuin1 in uterine cancers

Uterine endometrial cancers are often classified into two categories: (1) type-I, containing endometrioid carcinomas; and (2) type-II serous cancers, including clear-cell carcinomas (Goebel et al. 2018). The role of the cell cycle-affecting histone deacetylase Sirtuin 1 (SIRT1) has been investigated in multiple cancer types, with somewhat differing results obtained. For instance, in endometrial and ovarian epithelial cancers, an increase in its expression has been described (Asaka et al. 2015; Bartosch et al. 2016; Jang et al. 2009), correlated with improved overall survival in ovarian cancer, but lower survival rates in endometrial cancer. Beyer et al. (2020) have now investigated the immunohistochemical staining of SIRT1 in endometrial type-I carcinomas and clear cell carcinomas of the uterus in an attempt to correlate its expression with clinical parameters including patient survival. Their study cohort included 59 cases of endometrioid carcinoma and 6 cases of clear cell uterine carcinoma. They characterized immunohistochemical expression of SIRT1 with the Remmele immunoreactive score (IRS), which multiplies the level of staining intensity by the percentage of positively stained cells. They found that the expression of SIRT1 was significantly higher in the endometrioid carcinoma samples compared to those of clear cell carcinoma.

Fig. 2 Immunohistochemical detection of the nuclear NAD-dependent histone-deacetylase Sirtuin 1 is related to significant better progression-free survival in uterine endometrioid and clear cell cancer. From Beyer et al. (2020)

They also found that progression-free survival time was increased in those endometrial cancer patients with SIRT1 expression versus those showing no expression in the tumor (Fig. 2).

However, those endometrial cancer patients with SIRT1-positive staining showed significantly better progression-free survival than clear-cell cancer patients with SIRT1 expression. Moreover, the authors compared the expression of SIRT1 with sections from the same tumors previously stained with antibodies against the tumor suppressor proteins ARID1 A and β -catenin; they found a positive correlation between high SIRT1 expression and strong expression of both tumor suppressor proteins. The authors conclude that their results suggest that in uterine tumors SIRT1 may function as a tumor suppressor.

Testing behavior of human melanoma cells in CAM

The dorsal skin-fold chamber and its modifications (Asaishi et al. 1981; Alexander et al. 2008; Leunig et al. 1992) are commonly used to investigate growth behavior and invasion of cancer cells, their interaction with the adjacent stroma, and neoangiogenesis in vivo by various microscopic and immunolabeling techniques. Another useful model is the chorioallantoic membrane (CAM) of the chicken embryo which also permits microscopic observation in vivo (Ribatti 2014, 2016). In their present study, Strnadová et al. (2020) applied the CAM to analyze the growth behavior of three invasive human melanoma cell lines (A2058, BLM, and G361) either by analyzing whole-mount CAM or sections of FFPE specimens. Before seeding, the melanoma cell cultures were grown in medium conditioned with fibroblasts prepared from CAM or adult chicken skin, which significantly increased their proliferation compared to DMEM supplemented medium. Two days after seeding, the different melanoma cell lines formed distinct vimentin-positive colonies on the surface of CAM above the vessel layer as visualized by injected India ink or X-ray micro-CT (Fig. 3).

At day 6 after seeding, melanoma cell colonies had grown to a larger size and the vascular bed of the CAM was significantly changed, however without penetrating the tumor cell colonies. Furthermore, invasion of the CAM stroma by melanoma cells was only rarely observed (2/15 CAM) and no metastasis were formed in liver, lung and brain. The CAM vessels behaved differently in a hypoxic tissue niche model. Grafted isolated limb bud primordia were penetrated by CAM vessels and subsequently the grafted limbs developed well. Although the melanoma cells did grow around the grafted limb buds, they rarely invaded them (2/15). It is concluded that the CAM can be used as a system to study aspects of melanoma growth and behavior. Limitations are

Fig. 3 Chick CAM 2 days after application of BLM (**a**, **b**) and A2058 melanoma cells (**c**, **d**) after intravascular injection of vessels by India ink in bright and dark field mode (**a** and inset). The melanoma cells are positive for human vimentin (red in **b**, **c**). Nuclei of melanoma cells and CAM cells are stained blue by DAPI (**b**, **c**). The white dashed line in **b** indicates prominent vessels, which are also obvious by X-ray micro-CT following injection of phosphotungstic acid (**d**). From Strnadová et al. (2020)

the missing invasive and metastatic growth which may be related to the short experimental duration or unknown interfering properties of the CAM.

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News from the Society for Histochemistry

16TH INTERNATIONAL CONGRESS OF HISTOCHEMISTRY AND CYTOCHEMISTRY

Dear Colleagues,

In the light of the COVID-19 pandemic, the ICHC 2020 organizers and IFSHC Executive Council decided to postpone the ICHC 2020 to **5 - 8 September 2021**. The ICHC 2021 will take place as originally planned in the Cubex Centre, Prague, Czech Republic. The safety of all participants is our top priority. We are sorry for any inconvenience the postponement might have caused you.

The ICHC is held every four years under the auspices of the International Federation of Societies for Histochemistry and Cytochemistry (IFSHC), which continually strives to provide grounds for communication and cooperation among scientists all over the world in the areas of cyto- and histochemistry, cell and tissue biology, microscopy, pathology and other relevant fields.

The city of Prague, also known as the heart of Europe, provides easy access for scientists from all over the world. The congress venue, Cubex Centre Prague which offers technologically and visually unique space, promises to leave everyone with an unforgettable experience. Of course, Prague prides itself with its beautiful historical architecture, technical monuments, celebrated cafés, great food, and beer. This will be underlined by the ICHC gala dinner in the famous Art Nouveau Municipal House, and a free beer party organized in the premises of the Staropramen brewery.

We hope that you will join us in Prague to discuss together your latest achievements and that the venue will provide great opportunities for specialists at all levels of their career, bringing lots of opportunities for strengthening international collaborations. Special attention will be therefore given to the presentations of students. We also expect a rich commercial exhibition where new and emerging technologies will be presented.

We are delighted to inform you that the following speakers will present a lecture at the congress:

Stefan Hell, a Nobel Prize laureate, Max Planck Institute for Biophysical Chemistry, Germany (keynote speaker)

Alev Erisir, Department of Psychology, University of Virginia, USA

Toyoshi Fujimoto, Juntendo University, Nagoya, Japan

Hans-Joachim Gabius, Institute of Physiological Chemistry, Ludwig Maximilians University of Munich, Germany

Bożena Kamińska, Nencki Institute of Experimental Biology PAS Warszawa, Poland

Takehiko Koji, Department of Histology and Cell Biology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Ohad Medalia, Department of Biochemistry, University of Zurich, Switzerland

See you all in Prague, September 2021!

Hinke Multhaupt, President of the IFSHC
Klara Weipoltshammer, President of the Society for Histochemistry
Pavel Hozak, Chair of the Local Organizing Committee

Contacts

We will keep the current domain:

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If you have any questions about abstracts, please contact: abstracts@ichc2020.com

Other inquiries and comments about the conference, please contact: info@ichc2020.com

ANNOUNCEMENT**The Society for Histochemistry**

Invites scientists to apply for the 2021 Robert Feulgen Prize. The prize is awarded for an outstanding achievement in the field of histochemistry.

The contributions may be either towards the development of new histochemical and cytochemical techniques or in the application of existing technology towards solving important problems in biology and/or medicine. Addressed are scientists working in microscopical sciences (in the widest sense) as well as in biochemistry, cell biology, endocrinology, in situ molecular techniques, and neurosciences. Scientists in their mid-career (assistant or associate professor, priv. doz.) are encouraged to apply. The prize is not intended for lifetime contributions.

The Prize consists of a monetary prize of €2,000

All applications should be submitted before January 31, 2021 via the electronic submission system at: <https://www.greception.com/form-login-window/191a281d/>

The application should contain a short curriculum vitae, a 1,000 word summary of the contributions of the applicant and PDF reprints of the pertinent publications. Full description of conditions is available on the Society website: http://histochemistry.eu/description_of_conditions_.html

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