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REVIEW

Histochemistry and cell biology: the annual review 2010

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Abstract This review summarizes recent advances in histochemistry and cell biology which complement and extend our knowledge regarding various aspects of protein functions, cell and tissue biology, employing appropriate in vivo model systems in conjunction with established and novel approaches. In this context several non-expected results and discoveries were obtained which paved the way of research into new directions. Once the reader embarks on reading this review, it quickly becomes quite obvious that the studies contribute not only to a better understanding of fundamental biological processes but also provide use-oriented aspects that can be derived therefrom.

Keywords Cell biology · Histochemistry · Model · Stem cells · Cell organelles

Introduction

The year 2010 in the journal "Histochemistry and Cell Biology" is clearly marked by a multiplicity of excellent contributions touching many areas of the life sciences and rendering again pathbreaking insights into the structure, function and networks of biological systems. Many of the reports focused on molecules displaying a potential to be used as diagnostic tools, prognostic markers or therapeutic agents in diseases, inflammatory reactions and tissue recovery or which are serviceable as cyto- and patho-diagnostic cell-type and lineage markers or which can be exploited in cell sorting and purification strategies. Other studies alluded to cells practicable for therapeutic approaches or on target molecules useful for the development of gene or photodynamic therapy strategies. Also noteworthy are reports which focused on cell-/tissue-to-pathogen interactions. The present review intends to provide an insight into such exciting studies, but surely can only touch the surface of their width and depth. Hopefully, this approach will inspire the reader to delve into the primary literature.

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Central nervous system

The importance of differential expression and sorting of potassium channels and the development of a distinct ion channel profile for hippocampal network formation prompted Pruss et al. (2010) to determine histochemically temporal expression of potassium channels during mouse postnatal hippocampal development in vivo and in culture. With the focus on potassium channels displaying axonal sorting in diverse hippocampal neurons, it was observed that certain ion channels displayed considerable temporal variation of axonal localization among neuronal subpopulations. The authors conclude that age-dependent axonal sorting offers a new approach to functionally distinguish classes of hippocampal neurons and that this may contribute to the understanding of hippocampal network biology.

To be able to study striatal (caudate/putamen—CPu) neuronal networks, cultures need to be established, expressing the full compliment of interneurons and projection neurons in the proportions found in vivo. However, such cultures [typically derived from late embryonic day E18.5 (rat) and E14.5 (mouse)] were found to lack an important interneuron type, the giant aspiny cholinergic

neuron. Schock et al. (2010) overcame this problem through combining dissociated striatal neurons from an early (contains all interneurons) with that of a late (lacks cholinergic interneurons) gestational state. The later developmental date was required to yield the proportions of neurons found in vivo. With CPus containing or lacking cholinergic interneurons it was now possible to specifically examine the function of the cholinergic interneuron type in striatal networks.

Agmatine (decarboxylated L-arginine) plays several roles in mammalian tissues and generates anticonvulsant, antineurotoxic and antidepressant-like actions (Halaris and Plietz 2007). Due to the lack of enzymatically active preparations, information regarding to agmatine metabolism in the brain has been scarce. Therefore, Mella et al. (2010) took up work on a recently identified protein (agmatinase-like protein) that displayed agmatinase activity (Uribe et al. 2007). In the rat brain, the authors found that localization of this protein (restricted to the hypothalamus and the hippocampus) coincided with that described for its substrate. The results helped to explain reported agmatine-associated actions [in particular neurotransmitter/neuro-modulatory actions—(Reis and Regunathan 2000), but also learning and memory—(Liu et al. 2008)] in the brain, which the authors attribute to the activity of the agmatinase-like protein.

Cholesterol homeostasis is essential for CNS functioning and involves the cholesterol eliminating neuron-specific enzyme cholesterol 24-hydroxylase (Cyp46) (Lund et al. 1999; Ramirez et al. 2008). As so far only few studies demonstrated atypical Cyp46 expression in astrocytes under certain circumstances [e.g. human frontal cortex—(Brown et al. 2004), and advanced Alzheimer disease—(Bogdanovic et al. 2001)], Smiljanic et al. (2010) were interested to study cellular and temporal pattern of Cyp46 expression in the context of traumatic brain injury (TBI), a widespread cause of death and major source of adult disability. Employing a rat TBI model the authors found long-lasting neuronal and glial Cyp46 expression at the lesion site, indicating that TBI-induced production of damaged cell membranes elicits wide-spead Cyp46 expression to re-establish brain cholesterol homeostasis required for a glial scar formation.

Vascularization of the retina is driven through a transient period of hypoxia, induced by the metabolic demand of neurons and involves migration of hypoxia-sensing astrocytes which express vascular endothelial growth factor and guide the leading endothelial tip of vascular sprouts (Chan-Ling et al. 1995; Kim et al. 2006). Based on these observations, Kim et al. (2010) investigated neuronal-dependent foetal human retinal vasculature development of the retina of spontaneously aborted anencephalic (AnC) foetuses, which is devoid of ganglion cells and lacks blood vessels (Boniuk and Ho 1979; Hendrickson et al. 2006). The authors found in AnC retina localization of apoptotic cells primarily in the ganglion cell layer (compared to the retinoblastic layer in control retina), lack of expression of hypoxia-inducible factor-1α and reduction of retinal endothelial cells and astrocytes. It is suggested that the selective depletion of ganglion cells and therefore the loss of a substantial metabolic load is the basic cause of impaired vascular development in AnC retina.

Shoji et al. (2010) suggested, based on previous studies of expression of neurotransmitters receptors in neurons and satellite cells (SCs) of sensory ganglia (Hanani 2005; Julius and Basbaum 2001) that nodose ganglion (NG) neurons are regulated by surrounding SCs through glutamate and GABA and that such neuron–glia interactions may be involved in regulating viscerosensory informations from visceral organs. The authors found immunoreactivities for GLAST (glutamate transporter) and GAT-3 (GABA-selective transporter) in neurons and SCs. Failure of a bicuculline ($GABA_A$ receptor antagonist)-dependent increase in $[Ca^{2+}]_i$ in isolated NG neurons lacking SCs further supported the close functional association between neurons and SCs.

Kv3.1 and Kv3.3, voltage-gated potassium channels, highly coexpressed in granule cells (Ozaita et al. 2002; Sekirnjak et al. 1997; Weiser et al. 1994, 1995) play important roles in normal cerebellar function. Applying high-resolution immunocytochemical techniques for electron microscopy in the rat cerebellar cortex Puente et al. (2010) investigated precise ultrastructural localization of Kv3.1 (Kv3.1b) and Kv3.3 in specific compartments of parallel fibres (parallel fibre synaptic terminals (PFT) and intervaricose segments). Parallel fibres showed a higher percentage of labelling for Kv3.1b than for Kv3.3 with differences in labelling intensities. Noticeably, a high percentage of Purkinje cell dendritic spines were positive for Kv3.3, with a higher labelling density than in PFTs. It is concluded that the relative distributions of Kv3.1b and Kv3.3 greatly contribute to the molecular architecture of the granule cell parallel fibres.

Tissues yielded from *Bombyx mori* (*B. mori*—silk moth) were used by Uno et al. (2010) to determine expression of Rab protein family members Rab7 and 11. Ubiquitous presence was noticed for Rab11, whereas a tissue-specific occurrence (brain, testis and ovary) was seen for Rab7. In the brain, the Rab proteins were found in neurons of the dorsolateral (DL) areas, with Rab11 to be co-expressed in DL-neurons immunoreactive for PERIOD. With PERIOD, a protein associated with circadian rhythm and expression in neurons of the circadian system (Hall 2003) the Rab proteins are suggested to possibly play a role in the control of the circadian rhythm.

The lack of investigations as to a nutrient metabolic system within the retina provoked Atsuzawa et al. (2010) to perform studies on the localization of fatty acid β-oxidation enzymes in retinal tissue. Enzymes were found to preferentially localize to mitochochondria of Müller cells, principally to the endfeet of these glial cells, but were also detected in retinal neurons. Expression levels were, however, far less compared with those detected in the hepatic tissue.

Peripheral nervous system

The study by Brehmer et al. (2010) aimed at further clarifying neuronal network architectures in the submucosa of human intestines applying immunohistochemistry to submucosal whole mounts. The authors found two ganglionic nerve networks: a monolayered plexus submucosus externus with nerve fibres joining each other in acute or obtuse angles, which formed relatively wide meshes of often polyangular shape and a multilayered plexus submucosus internus. Communication between both plexus occured by interconnecting coiled strands involving sometimes intercalating ganglia. It is concluded that in the human submucosa of the small and large intestine, two ganglionic nerve networks exist and that Meissner (plexus submucosus internus) and Schabadasch (plexus submucosus externus) represent historically justified eponyms.

Han et al. (2010) performed studies on interstitial cells of Cajal (ICC) biology in the murine colon, applying immunohistochemistry with the Kit protein as ICC marker, Ki67 as marker for proliferation in conjunction with BrdU incorporation. Distinguishing between plexus myentericus-associated ICCs (ICC–MY), ICCs located at the border of circular muscle layer and submucosa (ICC–SM), within the smooth muscle layers (ICC–IM), and beneath serosa (ICC–SS), the authors could prove the existence of a proximal to distal and transmural gradient of ICC in the postnatal colon along with a dramatic increase of ICC cell number from neonatal to adult life. Increase of ICC cell number occured mostly from proliferation of ICCs. However, $Kit^{+}/Ki67^{+}$-ICCs were not found in ICC–SM throughout the postnatal period, indicating that increase of ICC–SM here occured exclusively through proliferation at the level of Kit-ICC–SM progenitor cells.

P2X receptors represent ligand-gated cation channels, of which the biology is largely determined by its subunit makeup. With respect to previous studies concerning expression of P2X receptor subunits P2X2, 3 and 5 in the gastrointestinal tract of various animals, Yu et al. (2010) extended work on the P2 × 4 and P2 × 6 receptors in the rat gastrointestinal tract. The authors observed in the submucosal and myenteric plexus intrinsic sensory neurons to be positive only for P2 × 6 receptor. As such receptors form only heteromultimers with either P2 × 2 or P2 × 4 receptors (North 2002). Yu et al. (2010) concluded that ATP, which acts on P2X receptors and plays a role of an excitatory neurotransmitter in the CNS, PNS (sympathetic neurons) and ENS (enteric ganglia) (Burnstock 2007; Dunn et al. 2001), is probably involved in regulating physiological functions of submucosal/myenteric plexus neurons through a heteromeric P2 × 6/2 receptor subunit makeup.

Stubinger et al. (2010) focused on the chemical coding of, and synaptic inputs to the intrinsic choroidal neurons in the chicken eye. Chicken were used as they represent an useful animal model of emmetropization and its underlying mechanism. The histochemical phenotype of nerve fibres within the choroid, and of the neurons in all ocular-related cranial ganglia, i.e. the trigeminal, ciliary, superior cervical and pterygopalatine ganglia were additionally addressed. With such studies, the authors wanted to prepare the basis for upcoming lesion and pharmacological experiments which should contribute to elucidate ICN functions and choroidal innervation patterns.

There is unequivocal evidence that up-regulation of chemokines and their receptors, including stromal cell-derived factor-1 (SDF1) and its CXCR4 receptor in dorsal root ganglia (DRG) may be one of the mechanisms directly or indirectly contributing to the induction and maintenance of neuropathic pain (Bhangoo et al. 2007; Oh et al. 2001; White et al. 2007). Using chronic constriction injury (CCI) of sciatic nerve (Bennett and Xie 1988) as an experimental model of neuropathic pain, Dubovy et al. (2010) investigated changes in SDF1 and CXCR4 expression post unilateral CCI of sciatic nerve in the ipsi- and contralateral DRG of cervical (C7–C8) and lumbal (L4–L5) segments. The authors found that unilateral CCI induced bilateral alterations of SDF1 and CXCR4 in L4–L5 and interestingly also in C7–C8 DRG, possibly mediated by interneurons in the spinal cord and at supraspinal levels, but humoral signalling by the bloodstream may also occur. However, the functional involvement of these alterations seen in DRG non-associated with injured nerve in neuropathic pain remains to be elucidated.

Detection of abnormal cellular prion protein (PrP^{Sc}) in lymphatic tissues and subsequently along neural projections to the CNS has led to the conclusion that these tissues are implicated in the spread of prion agent to the CNS (Kratzel et al. 2007). The enteric nervous system (ENS) of the gut is the first neuronal tissue where ingested PrP^{Sc} can be detected [e.g. (Andreoletti et al. 2000; van Keulen et al. 2000)]. PrP^{Sc} uptake and also the neuroinvasion process might be mediated through dendritic cells (DCs), which have been shown to establish neuroimmune interfaces with nerve fibres. Using an in vitro co-culture system of bone marrow DCs (BMDCs) and sensory neurons obtained from

dorsal root ganglia Dorban et al. (2010) could demonstrate that PrP^{Sc}-loaded BMDCs were competent to transmit PrP^{Sc} to the sensory neurons. Further studies are aimed to investigate the species barrier using BSE and vCJD strains or to test molecules that could be potential candidates to reduce or stop prion propagation.

Cochlea, organ of corti

A function of transient receptor potential channels (TRPC), which are known to display significant Ca^{2+} permeability, in cochlear sound transduction and neurotransmission is assumed by Tadros et al. (2010) due to (a) strong expression of TRPC3 ion channels in the spiral ganglion (SG) neurons, inner hair cells (IHC), outer hair cells (OHC) and the gap junction-coupled epithelial cells lining the scala media of adult guinea pig and mouse cochleae and (b) several correlations, such as Ca^{2+} entry mechanism in IHC and OHC of the guinea pig and rat cochlear attributable to TRPC3 expression (Raybould et al. 2007), similarities between TRPC3-specific signalling pathways in CNS neurons and those found in cochlear SG neurons, Ca^{2+}- and therefore TRPC3-dependent recycling of potassium in cochlear function (Jagger and Forge 2006; Spicer and Schulte 1998; Sun et al. 2005). The second study addressed TRPC3 expression during mammalian cochlear ontogeny (Phan et al. 2010). The authors observed prominent TRPC3 expression in differentiating epithelial cells of the cochlear duct, in the embryonic and early postnatal hair cells, but reduced expression once maturation of the cochlea structure had begun. TRPC3 labelling contrarily increased within cell bodies of cochlear/vestibular primary afferent neurons from the onset of hearing. Supported through previous observations, for example, that Ca^{2+} current peaks in the first postnatal week (Beutner and Moser 2001; Marcotti et al. 2003), which corresponds to establishment of synaptic transmission just prior to onset of sound transduction, it is concluded that spatiotemporal expression of TRPC3 is in line with the development of sensory, neural and epithelial cochlear tissues, as well as with hair cell Ca^{2+} homeostasis and regulation of auditory neurotransmission. The last work on cochlea biology concerns the expression of selected family members of ectonucleoside triphosphate diphosphohydrolases (E-NTPDases5 and 6), in the context of cochlear embryogenesis and early postnatal development of the cochlea (O'Keeffe et al. 2010). E-NTPDases play important roles in extracellular purineric P2 receptor signalling pathways. The authors detected NTPDase5 in developing sensory hair cells and supporting Deiters' cells of the organ of Corti during early postnatal period, whereas NTPDase6 was confined to the embryonic and early postnatal hair cell stereocilia. Both enzymes displayed colocalization with the UDP-preferring P2Y4, 6 and 14 receptors during cochlear development, in the adult cochlea; however, this colocalization was lost. Due to spatiotemporal topographic expression of these NTPDases and UDP-preferring P2Y receptors in the adult and developing cochlear a strong support for the role of pyrimidinergic signalling in cochlear development is assumed.

Lung

PLUNC (Palate, Lung, Nasal Epithelial Clone) protein family members [short (s) and long (l) PLUNC proteins], poorly described yet, are expressed in the upper respiratory tract and oral cavity where they fullfill a host defence function (Canny and Levy 2008). Proteomic studies have reported LPLUNC1 to be in nasal secretions, bronchoalveolar lavage and sputum (Casado et al. 2005; Nicholas et al. 2006; Wu et al. 2005) and to be a major secreted product of cultured tracheobronchial epithelial cells (Candiano et al. 2007; Kesimer et al. 2009). Immunohistochemistry was therefore applied to address expression of LPLUNC1 in tissues of the human respiratory tract, oro- and nasopharynx (Bingle et al. 2010). LPLUNC1 was found to be abundantly expressed in a population of goblet cells in the upper respiratory tract and was strongly expressed in airway submucosal glands and a range of minor glands present in the oral cavity and nasopharynx, suggesting a role for LPLUNC1 in mucosal surface protection at these sites.

Studies by Basset et al. (1987) and Boyd (1990) revealed dependence of lung liquid absorption in the rat on Na^+–glucose cotransport and assoziation of [3H]phloridzin, an inhibitor of the Na^+-dependent glucose transporter SGLT1, with alveolar type II cells (Boyd 1990). Based on such findings, Bodega et al. (2010) extended research on SGLT1 expression in the lung and found in the rat and lamb alveolar epithelium, that cells positiv for the *Erythrina cristagalli* lectin, a marker of alveolar type I cells, contained SGLT1. With alveolar type I cells providing 95–97% of the alveolar surface, the authors suggested that SGLT1 expression could be useful in lowering glucose concentration in the alveolar surface liquid to prevent pulmonary infection (Baker et al. 2006) and to contribute to a quicker removal of alveolar liquid in case of alveolar flooding.

Hermansky Pudlak syndrome (HPS), a heterogeneous recessive genetic disease which affects vesicle trafficking in lysosome-related organelles (e.g. lamellar bodies—Bonifacino 2004; Wei 2006) was the focus of studies conducted by Wang and Lyerla (2010). Such patients develop pulmonary fibrosis with ageing (Nakatani et al. 2000) and have alveolar type II (ATII) cells containing giant lamellar bodies. Studies on an HPS mouse strain which shows similarities with respect to dysmorphic ATII cells and contains

foamy alveolar macrophages (AMs) and on an HPS unrelated strain, also displaying dysmorphic ATII cells, revealed only in HPS mice age-dependent development of lung fibrosis together with the detection of high levels of alveolar TGF-β1 (known to contribute to collage production), which is attributed to activated AMs. The authors thus regarded HPS mice to be suitable to serve as a model for interventive therapy in HPS.

The sonic hedgehog (Shh) signalling pathway was central to investigations conducted by Zhang et al. (2010a), as it is crucial for the patterning of early lung morphogenesis in mice. It involves secreted, autocatalytically processed Shh, its receptor Patched (Ptch) (Chen and Struhl 1996), membranal Ptch-inhibited Smoothened and the Gli transcriptional factors (Thievessen et al. 2005). As the role of the Shh cascade in the developing human lung has not been determined, expression of Shh-signalling pathway components was addressed on human embryos of 7th–21st week of gestation. The studies revealed that mostly Shh-signalling pathway components in the developing human and mouse lung shared similar expression patterns, suggesting regulatory molecular networks during the early lung branching process to be conserved. The authors believed that studying Shh–Ptch–Gli signalling would contribute to a better understanding of the pathogenesis of Shh-related lung diseases in human and to the determination of potential therapeutic targets.

Heart and blood vessels

The studies of Toivonen et al. (2010) related to the analysis of expression pattern of receptors for several Adenoviral (Ad) serotypes in normal and dilated cardiomypathy (DCM) hearts. A particular focus was set on the commonly investigated Ad receptors, the coxsackie and adenovirus receptor (CAR) and CD46. The authors found in normal heart CAR expression to be confined to the subendothelial layer of intramyocardial coronary vessels, whereas in DCM, CAR expression changed to the endothelial cell layer. Similar observations were made for CD46. The authors thus regarded their result, i.e. the change of expression of CAR/CD46 receptors from subendothelial to endothelial layer of intramyocardial coronary vessels in DCM hearts, potentionally applicable for the development of targeted gene therapy for heart failure through intravascular administration of CAR/CD46 receptors targeting Ad viruses.

Berndt et al. (2010) studied the spatial relation of oncofetal fibronectin (Fn) and tenascin-C (Tn-C) isoforms (which have been shown to be reexpressed during tumour angioneogenesis and to display association with tumour tissues and vessel morphogenesis) to the stroma, vessels and vascular basement membranes of selected carcinomas. The results revealed deeper insights into the biology of tumour vessels (due to the differential distribution, vascular positivity and stratified organisation of oncofetal Fn and Tn isoforms), which could be benificial for the development and improvement of new angiogenesis-related cancer treatment strategies using Fn and Tn-C domain-specific antibodies as vehicles for vascular pharmacodelivery (using antiangiogenetic and antifibrotic agents) and tumour imaging (Neri and Bicknell 2005).

Amyloid-β (Ab) (considered to have a causal role in Alzheimer's disease) and the importance of its cerebral clearance across the brain blood barrier (BBB) were the main focus of the research conducted by Ueno et al. (2010). Expression of possible Ab-acting receptors in endothelial cells (the low-density lipoprotein receptor—LDLR, LDLR-related protein 1—LRP1 and the receptor for advanced glycation end products—RAGE; (Abdulkarim and Hameed 2006; Deane et al. 2003; Fryer et al. 2005; Sagare et al. 2007) was investigated in the hippocampus of stroke-prone hypertensive (SHRSP) rats. Such rats have recently been shown to display BBB impairment. Results revealed increased gene expression and protein levels only for LDLR in samples of SHRSP. Localization studies by immunoelectron microscopy found LDLR at luminal and abluminal cytoplasmic membranes and vesicular structures of endothelial cells as well as the cytoplasm of perivascular cells (presumably smooth muscle cells) in SHRSP and wild-type animals. The authors conclude that LDLR expression in SHRSP brains represents a protective response against a toxic stress load, such as Ab deposition, which is in accordance with a previous study showing inhibition of cerebral amyloid deposition and increased extracellular Ab clearance upon LDLR overexpression (Kim et al. 2009).

Cardiac-expressed caveolin Cav-1 [atrial cardiac myocytes and cardiac endothelial cells—(Gazzerro et al. 2010; Volonte et al. 2008)] and purinergic cell surface receptor/ATP-gated ion channel P2 $\times$ 7R, important in cardiac function (Gurung et al. 2009; Musa et al. 2009; Ralevic and Burnstock 1998, 2003; Vassort 2001) and shown to associate with pulmonal Cav-1 (Barth et al. 2008), were central to the work of Barth et al. (2010). It was reported that P2 $\times$ 7R (the classical glycosylated 80 kDa isoform and an additionally detected 56 kDa product) was similarly expressed as Cav-1 in the mouse heart. However, increased atrial expression was observed for the 80 kDa isoform in Cav-1 deficient mice and increased atrial Cav-1 expression in P2 $\times$ 7R deficient mice. The authors thus regarded the cardiomyopathy seen in Cav-1 deficient mice to be the result of disturbed crosstalk between Cav-1 and P2 $\times$ 7R.

Abundance and distribution of advanced glycation endproducts (AGEs) were analysed by Donaldson et al. (2010)

in the human myocardium. AGE formation and accumulation are most accelerated under diabetes mellitus (DM), thus contributing to various complications (van Heerebeek et al. 2008). AGEs may also play a role in other disease processes (such as isolated systolic hypertension (HTN)—(Kass et al. 2001; Zieman et al. 2007)]. Applying immunoelectron microscopy using an anti-carboxymethyl lysine (CML) antibody (CML is a major antigenic AGE structure) to localize and quantify CML in myocardial tissue obtained by epicardial biopsy during coronary bypass grafting (CBG), CML was found to be scattered throughout cardiomyocytes in all patients (independent of HTN or DM) and in endothelial cells. With CML being very prevalent in CBG patients, Donaldson et al. (2010) concluded that AGEs could play a role in abnormal cardiomyocyte function.

Franz et al. (2010b) looked at chronic cardiac allograft rejection (CCAR), which is represented by cardiac allograft vasculopathy (CAV) and fibrosis and zoomed into associated problems, i.e. intimal thickening and extra cellular matrix remodelling involving vascular smooth-muscle cells (VSMCs) and fibro-/myofibroblast (MyoFb). This was done by analysing expression and distribution of selected molecules (i.e. α-smooth muscle actin (α-SMA) as a VSMCs/MyoFb marker and alternatively spliced fibronectin (Fn) and tenascin (T) variants, which show disease-associated re-expression (Franz et al. 2009; Franz et al. 2010a, b; Gabler et al. 1996; Imanaka-Yoshida et al. 2002; Imanaka-Yoshida et al. 2001). Employing a heterotopic rat heart transplantation model of chronic rejection, the results revealed α-SMA to be a valuable marker to detect CCAR and a contribution of Fn and Tn variants to the development of chronic rejection following heart transplantation. It is concluded that targeting Fn and Tn variants might represent future concepts to prevent and treat heart-transplanted patients suffering from chronic rejection.

The process of autophagy, thought to be cardioprotective and to limit oxidative stress after an ischaemic insult (Rothermel and Hill 2008), has been suggested to contribute to reperfusion injury following restoration of blood flow to ischaemic myocardium. French et al. (2010) therefore investigated the extent of autophagy in hearts subjected to ischaemia with or without reperfusion. Using transgenic GFP-LC3 mice, subjected to coronary ligation, absence of autophagy-indicative GFP dots in infarct zones and a marked reduction of GFP dots in peri-infarct zones compared to sham-operated mice were observed, independent of reperfusion. Lack of appearance of autophagy-indicative LCII protein and upregulation of autophagy inhibitor mTOR were confirmative in this regard. The authors noted that pharmacological augmentation of autophagy may favourably modify the evolution of myocardial infarction induced by ischaemia followed by reperfusion, and that inhibition of mTOR with rapamycin (an inhibitor of mTOR) may prove most beneficial at least 24 h after onset of ischaemia, a hypothesis consistent with a recent report by Buss et al. (2009), showing reduced infarction sites and attenuation of negative remodelling in rats subjected to chronic ischaemia and rapamycin treatment 3 days post induction of ischaemia.

Kidney

Prominins (prominin-1 and prominin-2) represent a group of evolutionarily conserved pentaspan transmembrane, cholesterol-interacting glycoproteins, the function of which has not yet been elucidated. Prominin-1 (CD133) is expressed by somatic stem and progenitor cells and is found not only in the apical (luminal) side of various developing and adult epithelia including the kidney, but also in non-epithelial cells (photoreceptors and glial cells). In the kidney, prominin-1 has been detected as a brush-border bearing molecule of proximal nephron tubules and parietal layer of the Bowman's capsule (Weigmann et al. 1997; Florek et al. 2005). Devoted to such proteins, Jaszai et al. (2010) investigated spatial distribution of prominin-2 in the kidney. In contrast to prominin-1, prominin-2 was confined to the distal tubule and collecting duct. Both prominins were detected in human urine. These findings let the authors to conclude that prominins, due to their localization to distinct tubular segments, might be useful for novel diagnostic approaches, suitable to study renal diseases affecting respective parts of the nephron. Prominin's urinal presence might additionally offer a tool for diagnostic purposes of certain solid cancers, e.g. kidney cancer (Florek et al. 2005) or might be used to monitor functional kidney recovery upon tissue engineering/cell replacement therapies.

In the context of potential implications of molecular components of adherens junctions in cell signalling, Walter et al. (2010) investigated expression of p0071, the localization of which seems to occur exclusively to adherens junctions of simple and stratified epithelia, certain vascular endothelia and in composite junctions of intercalated discs of cardiomyocytes, in the kidney. Employing p0071-specific antibodies in conjunction with special markers for nephron segments, p0071-positive structures were predominantly identified as distal convoluted and straight tubules. In addition, p0071 was found in maturing nephrons of certain mouse developmental stages, which, due to a broader expression, prompted the authors to assume a role for this protein in kidney development. Furthermore, it is believed that p0071, due to its presence in distinct tubule segments and distal tubule cell-derived renal carcinomas, could be a cytodiagnostic marker in renal pathology.

Nurnberger et al. (2010) studied subcellular distribution of cell adhesion-associated proteins under conditions of human and experimental ischaemia, which are known to cause dramatic cellular changes (Bush et al. 2000; Molitoris 2004). Specimen from patients with and without acute kidney injury (AKI), a rat AKI model and proximal tubule-derived cells of opossum kidneys after ATP depletion-induced chemical hypoxia were used. In human and rat kidney, strong E-cadherin expression in distal tubule segments and N-cadherin expression restricted to proximal tubules was detected. In AKI and experimental ischaemia, depletion of only N-cadherin occurred, implicating a possible correlation between N-cadherin depletion and decreased cell adhesion, which together may possibly contribute to phenomena seen in AKI, such as back leak of the glomerular filtrate into the interstitium, detachment of proximal tubule cells and loss of epithelial polarity.

In kidney tubule cells, K^+ channels exert different functions, e.g. to avoid Na^+ influx-induced cell swelling, to maintain the cell's resting membrane potential, to retain Na^+ and avoid K^+ depletion during a restricted K^+ dietary intake and to secrete K^+ into the urine. Recent work identified the voltage-gated potassium channels Kv1.1, 1.3 and 1.6 in rat inner medullary collecting ducts (Escobar et al. 2004). The same group reported on voltage-gated ether-à-go-go-related Gene (*ERG*) K^+ channel isoforms in the rat kidney Carrisoza et al. (2010). Using an isoform-unspecific antibody, a quite heterogeneous ERG1 distribution was found with abundant expression in vessels and glomerular arterioles. Participation of ERG1 in membrane potential repolarization during Na^+ absorption was suggested, whereas vasodilator effects were assumed for EGR1 in glomerular arterioles and vessels, based on studies by Ohya et al. (2002) and Mewe et al. (2008).

A role for the stem cell factor (SCF)/KIT-signal transduction pathway in regulating the increase of ureteric bud branching and total number of nephrons in murine metanephroi has been suggested as well as in injured, inflamed and malignant kidney tissue, where upregulation of SCT and KIT levels has consistenly been observed. Based on such findings and the fact that mesonephric nephrons in ruminants are much more similar in certain aspects to those in humans, Tsikolia et al. (2010) investigated localization of SCF and its cognate receptor KIT in developing bovine meso- and meta-nephric kidney. The results implicated the participation of KIT in ureteric branching due to predominant localization of it at the ureteric bud tips and in advanced nephrons and in the development/differentiation of distal renal epithelia. With respect to SCF, restriction to the glomerular capsule and proximal tubule made SCF a specific marker for the proximal parts of the developing nephron. SCF/-KIT-signalling thus represents a general principle in kidney development. Previous studies by the same group identified KIT as a novel marker for foetal Leydig precursor cells (Tsikolia et al. 2009).

TRA-1-60 is a pluripotent stem cell indicator, involved in renal tubulogenesis (Kerjaschki et al. 1984; Cheng et al. 2005). Fesenko et al. (2010), therefore addressed its expression in human foetal, adult and diseased kidneys. TRA-1-60 was detected on ureteric bud and structures derived therefrom as well as in cells of collecting duct (CD) and thin limb of the loop of Henle of adult kidneys. A small population of TRA-1-60 positive cells was identified in the CDs and distal nephron structures of the outer medulla and in distal nephron compartments of renal cortex. In the cortex with tubulointerstitial injury, dramatic increase in the number of TRA-1-60, Pax-2 and Ki-67 (markers of tubular regeneration) expressing cells was observed. Taking this into account and that stem cells have not been identified in the distal nephron segment, aside from the papilla, being a nich for adult stem cells (Oliver et al. 2004), it is speculated that TRA-1-60 may identify a so far non-identified population of cells contributing to mechanisms of distal tubular repair.

The SLC26 family is a group of anion transporters (e.g. suphfate, oxalate), with family member SLC26A2 (DTDST) mediating sulphate transport. Due to the role of the kidney in maintaining sulphate balance, Chapman and Karniski (2010) performed localization studies of DTDST in this organ. DTDST localized to the microvillar region of rat cortical proximal tubule (PT) cells. As mice deleted for NaS_i1 [a Na^+-dependent sulfate cotransporter located in proximal tubule cells—(Lotscher et al. 1996)] displayed a 90% abrogation of Na^+-dependent sulphate transport, whereas Na^+-independent sulfate transport was unaffected (Dawson et al. 2003), a role for DTDST in a Na^+-independent mechanism to reabsorb filtered sulphate into the PT was suggested. Other studies implicated involvement of DTDST in oxalate-dependent NaCl reabsorption in this nephron segment, based on the sulfate dependency of this step and the fact that oxalacetat is a substrate of DTDST.

Liver

Fujii et al. (2006) recently reported, "cryobiopsy" followed by freeze-substitution fixation to be the best combination for paraffin-embedded tissue preparation (a) to antagonize artificial loss of glycogen and (b) to obtain strongest intensity by periodic acid-Schiff (PAS) staining. Taking this into account Saitoh et al. (2010) examined distribution of glycogen in livers of living mice under physiologic and fasting conditions applying PAS staining and α-amylase treatment to liver tissues obtained by cryobiopsy.

The authors could observe subtle differences in glycogen storage, with PAS-staining intensity around portal tracts more markedly reduced than at pericentral areas. Additionally, PAS-staining intensity directly around portal tracts was more markedly decreased than in the middle of zone I, indicating that zone I can be further separated into two tissue areas with respect to differences in glycogen production and consumption. The authors concluded that the technique applied might be applicable to the clinical evaluation of human liver tissues.

Using bile duct ligation as a model for hepatic fibrosis, Carvalho et al. (2010) explored the effect of bone marrow mononuclear (BMMN) cells, which are thought to participate in liver regeneration (Cantz et al. 2008; Gilchrist and Plevris 2010; Levicar et al. 2008; Sancho-Bru et al. 2009), post BMMN cell transplantation on the expression of marker proteins (i.e. laminin, cytokeratin-19, and alpha-smooth muscle actin (α-SMA)) for the cells (myofibroblasts) and the extracellularmatrix known to contribute to hepatic parenchyma remodelling. The authors could prove that BMMN cell injection had a diminishing impact on the numbers of myofibroblasts and the extent of the extracellular matrix, making such cells applicable to decrease hepatic fibrosis.

Metabolic zonation in the liver occurs through differential expression pattern along the porto-central axis. However, the proceedings behind are still not fully understood. The recent observation of a role of the Wnt/β–catenin signalling pathway in this process (e.g. (Benhamouche et al. 2006; Braeuning et al. 2007; Braeuning and Schwarz 2010; Giera et al. 2010; Tan et al. 2006) prompted Braeuning et al. (2010) to study the phenotype and growth behaviour of a number of residual β-catenin-positive mouse hepatocytes in a hepatic β-catenin-negative environment. The results clearly indicated that the zone-specific expression profile of hepatocytes is a process dependend on β-catenin.

Pancreas

Research on manserin was the focus of Tano et al. (2010). Manserin, derived from precursor molecule secretogranin II (SgII), belongs to a group of acidic, secretory proteins of endocrine, neuroendocrine and neuronal cells, which are referred to as chromogranins, secretogranins or granins. The authors used the pancreas, as it has been shown to be a source of SgII-derived peptides (Fischer-Colbrie et al. 2005; Leitner et al. 1996; Stridsberg et al. 2008; Trandaburu et al. 1999). Insulin-secreting β-cells and somatostatin-secreting δ-cells were found immunoreactive for manserin. So far the physiological relevance of manserin is still enigmatic. However, as Sg II-related peptides have been proven to be useful as possible tumour markers, Tano et al. (2010) see a potential of manserin as a diagnostic and prognostic marker for endocrine tumours (Conlon 2009).

Gouyer et al. (2010) performed a thorough characterization of the mucin MUC6 in wild-type and also in *Cftr*-deficient mice. Beside detection of MUC6 in various tissues and cells (deep glands of duodenum and ileum and mucous neck cells of gastric body, surface of the stomach and renal collecting tubules) staining was also found in centroacinar cells of pancreatic tissue. In *Cftr*-deficient mice higher expression of MUC6 was detected. As MUC6 is a significant component of material obstructing the small intralobular ducts in the pancreas of cystic fibrosis patients (Reid and Harris 1998) the authors believe that the Muc6-specific antibody, which seems highly specific to the mouse mucin, will be useful to study pancreatic pathology in cystic fibrosis.

Regulation of insulin release in pancreatic β-cells centrally involves calcium in conjunction with calcium-binding protein (CaBPs) dependent intracellular calcium-signalling pathways. In their study, Bazwinsky-Wutschke et al. (2010) investigated the occurrence of selected CaBPs in pancreatic tissues of non-diabetic rats and diabetic rats which represent the best characterized animal model of spontaneous non-insulin-dependent type 2 diabetes mellitus—NIDDM (Abdel-Halim et al. 1993; Frese et al. 2007; Ostenson et al. 1993) and also in rat insulinoma cells. The authors observed differences in CaBP distribution and also significantly higher transcript levels of all CaBPs in pancreatic tissues of diabetic rats. The authors, thus believe that such cellular alterations of CaBPs have important consequences regarding the maintenance of normal β-cell functions and therefore represent a central component in NIDDM.

Adrenal gland

Transcripts for all protein 4.1 homologs (4.1R, 4.1G, 4.1N and 4.1B, of which 4,1R has been extensively studied in red blood and nucleated cells) are abundantly expressed in the adrenal gland (Parra et al. 2000; Parra et al. 1998; Walensky et al. 1999; Walensky et al. 1998). Re-performing a more comprehensive subcellular analysis of all 4.1 protein isoforms and alternative spliced isoforms thereof in the adrenal gland of mice, Wang et al. (2010) observed different subcellular localizations (membrane, cytoplasm, perinuclear), indicating key roles for the 4.1 family in this endocrine organ. Through domain-specific interactions with deverse receptors and channels (Baines 2009; Binda et al. 2002; Fukatsu et al. 2004; Lu et al. 2004), a possible involvement in hormone secretion through interactions with yet-to-be-identified membrane proteins might be conceivable.

Epithelium

Interactions between melanoma, the most aggressive and deadly form of skin cancer, and the epidermal tumour microenvironment (ETM)—the multilayered epithelium of the skin—are poorly understood. In this regard, induction of connexins 26 and 30 (Cx26 and Cx30) in the epidermis adjacent to malignant tumours (e.g. melanoma and Merkel cell carcinoma), but not in the epidermis adjacent to benign tumours (e.g. melanocytic nevi and angiomas), has recently been demonstrated (Haass et al. 2003, 2006). In this context, Haass et al. (2010) found several correlations between (a) tumour thickness and vertical Cx26 and Cx30 expression, (b) tumour thickness and horizontal Cx26 dissemination, (c) metastasis and horizontal Cx26 expression and (d) vertical epidermal expression patterns of Cx26 and Cx30 and the proliferative index in the ETM. The authors thus could provide evidence for the association of ETM alteration with tumour malignancy and progression.

Miyazawa et al. (2010) addressed in their work the issue of bovine spongiform encephalopathy (BSE) due to spread of the BSE agent to the cattle population through the intestinal uptake of the agent from fodder containing meat and bone meal from BSE-infected brain or spinal cord (Mabbott and MacPherson 2006; Novakofski et al. 2005). As a step forward to be able to study pathogenesis of BSE and other bovine gastrointestinal diseases, the generation of a bovine intestinal epithelial (BIE) cell line would be quite advantageous. Miyazawa et al. (2010) established a permanent BIE cell line to be used as an in vitro M cell model. The most important characteristic of this cytokeratin-positive, adherence and tight junction forming cell line is the ability for transcytosis of macromolecules under certain circumstances. The authors thus regard the BIE cell line to be useful for studying transport mechanisms of various pathogens and also for the evaluation of drug delivery via M cells.

Chloride channels, calcium-activated (CLCA) proteins belong to a large protein family, the functions of which are poorly understood. They have been proposed to mediate calcium-activated chloride currents. CLCA proteins are widely distributed in mammalian tissues, but with a specific cellular expression pattern for each family member. However, some CLCA family member, such as human CLCA2 (hCLCA5) display a complex and partly contradictory expression pattern (epithelial but also endothelial cells). In this regard, Braun et al. (2010) began to systematically address the expression pattern of mCLCA5 (the murine ortholog to hCLC5). MCLCA5 was found virtually exclusively in keratinocytes of all stratified squamous epithelia that undergo cornification (including cornifying Hassall's bodies). Intracellularly, mCLCA5 localized to cytoplasmic granules of granular layer keratinocytes and, to a lesser extent, to spinous and cornified layer keratinocytes. Surprisingly, no membranal association of this protein was observed. Based on their findings, the authors suggested a role of mCLCA5 in maturation and keratinization of squamous epithelial cells.

In the human skin, stem cell compartments for the interfollicular epidermis (IFE) and the hair follicle (HF) are localized in the basal epidermal layer (Ghazizadeh and Taichman 2005; Lavker and Sun 1982, 2000) and within the bulge of the HF (Montagna 1962), respectively. Jiang et al. (2010) applied immunohistochemistry to whole mounted single HFs from the human scalp skin to search for stem cell markers suitable to distinguish basal IFE from bulge stem cells. They identified several markers to localize uniquely to the basal IFE (CD34, CD117), the bulge region (CD200) or both, basal IFE and the bulge region (CK15, CD49f, CD29). Other marker molecules were negative in this regard (CD71, CD24). The authors suggested usage of the identified markers in cell sorting and purification strategies, although some obstacles (e.g. CD200-based FACS sorting of bulge stem cells has not been so far successful) need to be tackled.

Chermnykh et al. (2010) developed a 3D model of epidermal morphogenesis, applying a new method of isolating dermal papilla (DP) cells, in order to gain informations on early stages of hair follicle development and signals governing this process. They demonstrated DP cells (as well as DP conditioned medium) to be able to induce the formation of multicellular tubule-like outgrowths of keratinocytes in the living skin equivalent in vitro. The authors could also overcome the problem of the loss of hair follicle-inducing ability and growth activity after several passages through the expression of lentiviral-based human telomerase reverse transcriptase. Such immortalized DP cells and also conditioned medium therefrom induced tubulogenesis in the 3D model after prolonged cultivation, although the process of immortalization of DP cells resulted in a diminished differentiation potential. However, the authors consider their model useful to study a large range of problems pertaining to epithelial, i.e. hair follicle—mesenchymal (i.e. dermal papilla) interactions.

The study by Garreis et al. (2010) extended research on human β-defensins (hBD), important antimicrobial molecules expressed at mucosal surfaces and other cell-types. Observations with respect to pathogens and proinflammatory cytokines (IL-1/TNF), able to stimulated hBD production (McDermott 2004) and vice versa, prompted the authors to investigate expression (at the ocular surface and lacrimal apparatus) and inducibility (through proinflammatory cytokines/ocular pathogens) of hBDs. Mouse BD were used to study ocular pathogen-induced expression in an ocular surface scratch model. HBD1-4 were constitutively expressed in conjunctival epithelial cells, IL-1 and *Staphylococcus aureus* were found to increase hBD2 and

hBD2/hBD3 expression, respectively, in cultured human corneal and conjunctival epithelial cells. The scratch model revealed mBD production only in the presence of a defective corneal epithelium. The authors regard hBDs to potentionally represent antimicrobial peptide-based therapeutic agents to deal with inflammatory reactions of the eye.

Molecules

Lu et al. (2010) identified a domain in exon 1B1b to be responsible for cytoplasmic retention of microphthalmia-associated transcription factor (Mitf), which has been shown to play regulatory roles in several hematopoietic cells (Rohan et al. 1997; Roundy et al. 1999; Stechschulte et al. 1987; Thesingh and Scherft 1985) as well as in melanocytogenesis, retinal pigmented epithelium development and late differentiation stage of osteoclasts (Steingrimsson et al. 2004). The authors suggested that the mechanism behind 1B1b-dependent nuclear entry possibly involves intramolecular NLS-masking (Bronisz et al. 2006; Lu et al. 2010). As M-CSF and RANKL stimulation of macrophages lead to phosphorylation (Mansky et al. 2002; Weilbaecher et al. 2001) and nuclear localization of Mitf in macrophages (Bronisz et al. 2006), Lu et al. (2010) assumed that such cytokines promote release of Mitf from its intramolecular NLS-masking mechanism with subsequent entrance of Mitf into the nuclear compartment.

Tanaka and Kamitani (2010) focused on the subcellular localization of Ro52, a RING (Really Interesting New Gene) finger domain (a type of zinc finger)—containing E3 ubiquitin ligase (Wada et al. 2006a) and a well known autoantigen associated with several autoimmune diseases (especially Sjögren's syndrome and systemic lupus erythematosus). The authors reported that Ro52-containing cytoplasmic structures, referred to as cytoplasmic bodies (Campbell et al. 2007; Reymond et al. 2001; Rhodes et al. 2002; Wada et al. 2006b; Yamauchi et al. 2008) were neither of mitochondrial origin nor did they represent proteasome-enriched structures, caveolae, endosomes, or lysosomes but displayed dynamic microtubular associations. Further research by Tanaka et al. (2010) revealed non-mutually exclusive interaction of Ro52 with Daxx and FLASH, two apoptosis-related proteins. With Daxx being mostly a nuclear localizing protein, the authors could demonstrate that overexpression of Ro52 and FLASH led to relocation of Daxx into the cytoplasm. However, as Ro52 did not act as an ubiquitinating enzyme on Daxx and FLASH the role of this heteromeric protein complex remains obscure.

An ultrastructural analysis of the nucleolar spatial arrangement of actin and nuclear myosin I (NMI) was performed by Philimonenko et al. (2010) to derive functional implications. The results were in line with the dynamic behaviour of NMI and its role in rDNA transcription (Percipalle et al. 2006; Philimonenko et al. 2010), through concentration of NMI mainly in the dense fibrillar component (DFC) of actively transcribing nucleoli and co-localization with nascent transcripts. However, the studies also revealed, due to the observation of an NMI fraction that did not associate with rDNA transcription, additional roles of NMI in other nucleolar processes (e.g. rRNA maturation or ribosome subunits transport). With respect to actin, co-localization with nucleolar factor UBF and RNA polymerase I independent of transcription and mainly in fibrillar centres (Fcs), transcriptionally inactive regions of the nucleolus, was observed. Apart from functioning in transcription elongation (Fomproix and Percipalle 2004; Philimonenko et al. 2004), the authors suggested an involvement of actin in the maintenance of nucleolar architecture.

Tumour progression due to disrupted intercellular junctions was the theme of the studies conducted by Phan et al. 2010. Several observations, e.g. the association of development and progression of primary tumours with aberrant signalling pathways of the hepatocyte growth factor (HGF) and its receptor c-Met (Lesko and Majka 2008), the correlation between matrix metalloproteinase (MMP)-7 overexpression and metastatic progression of hepatocellular carcinoma (Gao et al. 2006), HGF-induced MMP-7 expression in stomach cancer cells (Lee et al. 2007) or HGF/c-Met-dependent activation of the Wnt/β-catenin pathway (Apte et al. 2006) prompted the authors to investigate these entanglements in more detail. They found the HGF/c-Met/β-catenin/MMP-7/E-cadherin axis to be directly involved in HepG2 cell scattering and concluded that any therapeutic strategy disrupting this axis could represent a potential therapy candidate in HCC treatment.

A nucleocytoplasmic shuttling protein, Yes-associated protein 1 (YAP1), linked to cell contact inhibition, organ size control and cancer development and fitted with oncogenic and tumour suppressor functions, was addressed by D'Addario et al. (2010). YAP1 interacts with proteins critically involved in regulating the balance between proliferation (Danovi et al. 2008) and differentiation (Dellambra et al. 2000). To extend knowledge of YAP1's role in such processes its overexpression in human keratinocytes was studied. The authors found that YAP1 contributed to cell proliferation and immortalization, but malignant transformation was not observed. The authors concluded that overexpression of YAP1 seems to maintain keratinocytes in a proliferative state.

The observation that melatonin enhances in vitro differentiation of osteoblasts and promotes bone formation in vivo [e.g. (Nakade et al. 1999; Roth et al. 1999)] encouraged Kumasaka et al. (2010) to investigate involvement of

melatonin in tooth development and growth by immunolocalization of Mel1aR, the most potent transmembrane receptor for melatonin, using tooth germs from humans (late bell stage) and neonatal mice. They found Mel1aR to be expressed in secretory ameloblasts, the cells of the stratum intermedium and stellate reticulum, external dental epithelial cells, odontoblasts and dental sac cells. The authors assumed that melatonin may regulate proliferation, differentiation and/or function of odontogenic cells in the tooth germs to maintain the expanding tooth morphology. Of particular interest in this context is the observation by that the formation of prismatic enamel with cross striation occurs under the regulation of the circadian clock (Smith 2006).

Pressure-dependent ischaemia and hypoxia of the periodontal ligament (PDL) due to continuous orthodontic force on the tooth [e.g. (Macapanpan and Weinmann 1954; Reither 1960)] is a stimulus for expression of heat shock proteins (HSPs). This led Arai et al. (2010) to investigate quantitatively the role of HSPs during experimental tooth movement. The authors found time-dependent differential gene expression in the pressore zone of laser microdissected PDL, with the HSPA1A gene displaying initial strong expression, but decreased expression during the course of tooth movement. Based on the structural changes (compression and circulatory disturbance of PDL during tooth movement) followed by hypoxia in the pressure zone, the authors considered upregulation of HSPA1A as adaptation to the new environment to prevent initiation of apoptosis (Beere et al. 2000; Giffard et al. 2008; Saleh et al. 2000), whereas decreased expression of HSPA1A is finally the result of a loss of periodontal cells, due to hypoxia- and compression-induced apoptosis (Hatai et al. 2001), degeneration, inflammation (Krishnan and Davidovitch 2006; Saito et al. 1991) and osteoclastic bone resorption of the PDL.

Ca^{2+}-sensitive/calcineurin-regulated NFAT (nuclear factor of activated T cells) encompasses a multigene family, whose nuclear sequestration of subtype NFATc1A within heterochromatin containing foci upon repetitive skeletal muscle activity has been explored by Shen et al. (2010). As such foci did not colocalize with NFAT target genes [i.e. the slow and the fast myosin heavy chain genes—(Liu et al. 2001)] and did not seem to represent locations of active genes or RNA processing, but display high dynamic and rapid redistribution of foci-localizing NFATc1, it is assumed that such foci (a) retard nuclear export sequence-mediated efflux of NFATc1, in order to prolong transcriptional effects of NFATc1 (that entered the nucleus during prior muscle activity) and (b) allow rapid mobilization of foci-sequestered NFATc1 to other NFATc1-dependent nuclear sites.

The focus of the research conducted by Sisto et al. (2010) was to study the proteins furin, TACE (tumour-necrosis-factor-converting-enzyme), TNF-α and AREG (Amphiregulin) in biopsies derived from Sjögren's syndrome (SS) patients. SS is one of the most common autoimmune diseases (Boras et al. 2004; Cuello et al. 1998; Fox et al. 1994) associated with lymphocytic infiltration into salivary and lacrimal glands (leading to dry mouth and dry eyes) and altered production of the pleiotropic and pro-inflammatory mediator TNF-α (Boras et al. 2004; Cuello et al. 1998; Fox et al. 1994). TNF-α, if overexpressed, gives often rise to chronic autoimmune disorders and, in combination with IFN-γ, plays a role in the presentation of autoantigens, recognized by autoantibodies in many SS patients (McArthur et al. 2002). All proteins were found to display strong positive histochemical staining compared with control biopsies. The authors concluded that changes in furin, TACE and AREG expression could partly be responsible for pro-inflammatory cytokine (TNF-α) overexpression characterizing SS.

Suntornsaratoon et al. (2010b) reported for the first time on the chondroregulatory action of prolactin (PRL) on the growth plate of long bones in the context of lactation, using tibiae of rats at midpregnancy until 15 days postweaning. The study was based on previous observations, showing complex PRL-dependent maternal bone changes during reproductive periods (Seriwatanachai et al. 2008; Suntornsaratoon et al. 2010a). The authors found PRL receptor expression in lactating and non-lactating rats within the growth plate cartilage restricted to chondrocytes of the proliferating, hypertrophic and resting zone. PRL-dependent bone length increase (Suntornsaratoon et al. 2010a) correlated negatively with the total growth plate height during pregnancy and lactation, which, however, occured within different timeframes and was also PRL-dependent but pituitary PRL-independent.

The impact of retrotransposition events on angiogenic properties of endothelial cells, primarily tumour vascularisation, was studied by Banaz-Yasar et al. (2010) using expression of the abundant mammalian non-long-terminal-repeat (non-LTR) retrotransposon (LINE-1—L1) in a porcine aortic endothelail (PAE) cell model and various tumour tissues. For the first time, LINE-1 retrotransposition in vascular endothelial cells was observed, resulting in a reduction of endothelial cell proliferation and migration. Together with the observation that ORF2p, a nuclear acid-binding protein encoded by the open reading frame 2 of LINE-1, was almost not detectable in endothelial cells of tumour blood vessels, but in endothelial cells of human blood vessels, a potential link between LINE-1 retrotransposition events in endothelial cells (Ergun et al. 2004) and vascularisation of tumour tissue, by suppression of the angiogenic capacity of vascular endothelial cells was assumed.

Na^+/H^+ exchanger regulator factor 1 (NHERF1) contributes to the maintenance of the intestine ultrastructure (Kreimann et al. 2007; Morales et al. 2004). In mammary carcinoma cell lines and epithelial cells of the bile duct in rat liver, its expression is regulated by estrogens. Due to observations that NHERF1 may act as a tumour suppressor or as oncogene (Pan et al. 2006; Shibata et al. 2003) and that treatment with estrogens prevents colon carcinogenesis, a potential role of NHERF1 in colon carcinogenesis needed to be determined. Cuello-Carrion et al. (2010), therefore used the colon and uterus of cycling rats to study NHERF1 expression during the estral cycle. During the estrous stage the authors found oestrogen-dependent expression of NHERF1 in the colon. With their results the authors established the baseline for future studies regarding the role of NHERF1 in colon carcinogenesis.

Organells

To find out whether zebrafish represent a valuable model for evaluation of peroxisomal functions, distribution pattern of such organells in developing and adult zebrafish was determined by Krysko et al. (2010). The authors detected peroxisomes in the liver, the pronephric duct and the wall of the yolk sac in 4-day-old embryos, whereas prominent detection of catalase activity in adult zebrafish was observed in hepatocytes, the renal proximal tubules and the intestinal epithelium. Although peroxisomal distribution in zebrafish resembled the pattern in mammals, it was not possible to eliminate peroxisomes by morpholino oligos targeting peroxins, essential proteins for the biogenesis of peroxisomes, or through the expression of a potential dominant negative peroxin. The study thus demonstrated that other approaches are required (e.g. transgenic fish) prior usage of this fish species in peroxisome research.

Rab5(Q79L)-induced giant endosomes, distinguished by different microdomains and therefore useful to study intraendosomal protein localizations, were investigated by Wegner et al. (2010) applying a combination of confocal, time-lapse and electron microscopy. The authors observed that apart from their size, enlarged Rab5(Q79L) endosomes were ultrastructurally similar to normal endosomes, mostly having a morphology approximating early endosomes, multivesicular endosomes, lysosomes or a combination of these organells. However, recycling of the transferrin receptor was detected to be reduced as well as disturbed sorting of the EGF receptor. The results thus revealed the limitations of this system in endosome research considering the sorting defects of two representative receptors. However, the fact that Rab5 (Q79L) did not impede biogenesis of intraluminal vesicles in endosomes makes giant endosomes useful as a model to elucidate the mechanisms and requirements for intraluminal vesicle formation.

Correlations between function and shape of the cell nucleus were examined by Langevin et al. (2010) based on previous studies showing tissue stretch-induced cell spreading, lamellipodia formation and perinuclear α-actin redistribution (Langevin et al. 2005; Storch et al. 2007). Now Langevin et al. (2010) wanted to know whether tissue stretch might result in nuclear remodelling, measurable as a change in the degree of nuclear concavity. The authors observed that fibroblast nuclei from stretched tissue had a larger cross-sectional area, a smaller thickness in the plane of the tissue and smaller relative concavity. This was attributed to an active reorganization process because the stretch-induced change in nuclear shape could be prevented by Rho kinase inhibition suggesting involvement of actin-based cytoskeletal mechanisms. Cytochalasin-D treatments gave similar results (Deguchi et al. 2005; Guilak 1995). It was concluded that increasing nuclear convexity may have important influences on gene expression, RNA trafficking and/or cell differentiation.

Lack of a detailed knowledge of holo-HDL particle uptake provoked Rohrl et al. (2010) to look at the uptake and processing of HDL particles in HepG2 cells applying apoprotein labelling in conjunction with light and electron microscopy. The authors identified multivesicular bodies (MVBs) as main endocytic compartments. Early endosomes were rarely HDL-positive, and with MVBs being as early as 15 min HDL-positive, a rapid transfer process post HDL-endocytosis was indicated. Interestingly holo-HDL particles were only to a minor degree transported to lysosomes, suggesting that MVBs constitute a temporary storage compartment. As close Golgi apparatus associations of HDL-positive MVBs-appendices were observed, exchange of HDL-derived cholesterol from MVBs directly toward the Golgi apparatus was suggested.

Soriano et al. (2010) investigated possible internalization mechanism of liposome-based zinc(II)-phthalocyanine (ZnPc), a widely used photosensitizing drug (PS), using inhibitors of main endocytosis pathways, i.e. cytochalasin D (CD), a macropinocytosis inhibitor and dynasore (Dyn), an inhibitor of clathrin-mediated endocytosis—(Macia et al. 2006; Peterson and Mitchison 2002). This study in insofar of importance as PSs are widley used in photodynamic therapy of cancers and knowledge of mechanism of PS uptake is of paramount importance. The authors found only Dyn to alter photodynamic effects of ZnPc, and suggest that clathrin-mediated endocytosis is the main mechanism by which ZnPc is preferentially internalized into cells.

Caveolae, playing roles in many cellular functions, are typically described as omega-shaped invaginations of the plasma membrane in ultrathin sections of aldehyde-fixed cells. As only cryofixation methods are able to preserve the

plasma membrane (Ebersold et al. 1981; Kirschning et al. 1998; Szczesny et al. 1996), Schlormann et al. (2010) investigated the freeze-fracture behaviour of caveolae in glutaraldehyde-fixed and cryofixed mouse fibroblast cells. The authors found in rapid frozen cells without any chemical fixation all degrees of invagination from nearly flat, deeply invaginated to cup/flash-like. It is concluded that the constricted "neck" of caveolae (which makes the caveolae omega-like shaped) in most cases is a chemical fixation-induced effect.

Mammalian target of rapamycin (mTOR), a serine–threonine kinase involved in multiple cellular functions, is the catalytic subunit of two molecular complexes, mTORC1 and mTORC2, of which mTORC1 has previously been shown to display lysosomal targeting, a step dependent on amino acids and necessary for activation (Avruch et al. 2009; Hara et al. 1998; Sancak et al. 2008). As aberrant signalling of mTORC1 has been implicated in many diseases (e.g. cancer and diabetes) studies on the subcellular distribution of mTOR and associated proteins were conducted by Ohsaki et al. (2010b) using the mTOR inhibitors rapamycin and Torin1. The authors found that despite inhibitors mTOR and associated proteins were still able to be recruited amino acid-dependent to lysosomes, but to a greater extent than in the absence of inhibitors. Lysosomes thus harboured both active and inactive forms of mTOR and that the lock of mTORC1 onto the lysosomal membrane potentially caused the prominent accumulation of inhibited mTOR at this compartment.

Stem cells

Adipose-derived stem cells (ASCs) were re-subjected to studies previously conducted by (Ashjian et al. 2003) to re-evaluate the in vitro transdifferentiation potential of such cells into early neural progenitor cells (Kompisch et al. 2010). Applying a neurogenic induction protocol according to Ashjian et al. (2003), a characteristic morphology comparable to neuronal progenitors was partly seen, as well as expression of respective marker proteins. Reduced cell proliferation and G2-cell cycle arrest (a precondition for the neural cell fate determination seen in *Drosophila*—(Negre et al. 2003)] were also detected. Although the results pointed to a potential neurogenic transdifferentiation of ASCs into early neuronal progenitors, the authors emphasized the possibility that the neuron-like morphology could possibly be the result of the cell cycle arrest, rather being a characteristic of a specific transdifferentiation process.

Recent studies indicated that the human dental pulp from adult teeth contains adult stem cells (dental pulp stem cells—DPSCs). Ishikawa et al. (2010) expanded research on DPSC biology searching for putative stem cells (identified as slow cycling long-term label (BrdU)-retaining cells (LRCs) and Hoechst-effluxing, non-lineage marker-expressing cells [side population (SP) cells] in the pulp tissue of born rats. The authors found dense (i.e. high BrdU-labelling) LRCs, which postnatally decreased. 4 weeks after birth, LRCs resided in the centre of the pulp and associated with blood vessels. In the mature dental pulp <0.76% SP cells were identified, which included dense LRCs. Addressing the regenerative capacity of LRC after tooth injuries the authors observed that differentiating odontoblast-like cells contained LRCs, indicating that such cells represent DPSC in mature pulp tissues and play a role in the pulpal healing process.

Dental pulp-derived mesenchymal stem cells (DPSCs) have become increasingly popular to study as they are easily to obtain and propagate and display characteristics similar to other mesenchymal stem cells (MSCs). For the first time, Karaoz et al. (2010) were able to isolate and characterize DPSCs derived from human natal dental pulp (Hndp–SCs). Such SCs were compared with human bone marrow (hBM) MSCs. They observed that hNDP–SCs like hBM–MSCs expressed markers for glial and neuronal, myogenic, osteogenic, adipogenic and chondrogenic differentiation and could differentiate into such lineages. The authors proved that, beside small variations (hNDP–SCs are more developed and metabolically active and have a higher proliferation capacity, probably due to their anatomical localization), no significant differences were detectable between hNDP–SCs and hBM–MSCs.

Immunesystem

The observation that CNS infiltrates in experimental autoimmune encephalomyelitis (EAE—an animal model for multiple sclerosis) consist mainly of neutrophils (Maatta et al. 1998) and that depletion of peripheral blood polymorphonuclear leukocytes delayed or even prevented the development of EAE in SJL mice (McColl et al. 1998) prompted Wu et al. (2010) to investigate a possible entanglement of neutrophils in EAE pathogenesis. The authors found a spatiotemporal shift of neutrophils (mainly found in the meninges at onset stage, whereas parenchymal invasion occured at peak stage) and areas with demyelination, loss of axons and axonal degeneration containing a prominent accumulation of neutrophils in the spinal cord of EAE-induced C57BL/6 mice. The authors could thus prove the involvement of neutrophils in EAE progression and a contribution to tissue injury in the acute phase of EAE.

Activated dendritic cells (DCs) were shown to be able to penetrate gut epithelial monolayers to directly take up antigen without compromising the barrier function, to express tight junction molecules and establish tight junction-like

structures with adjacent epithelial cells (Rescigno et al. 2001). This provoked Kamekura et al. (2010) to investigate regulation of expression of tight junction molecules in a DC line. With studies being performed in the presence of DC-activating epithelial-derived thymic stromal lymphopoietin (TSLP - triggers DC-mediated Th2-type inflammatory responses) and toll-like receptor (TLR) ligands, the authors found in particular dose-, time- and NF-κB inhibitor (IMD-0354)-dependent upregulation for claudin-7. It is concluded that preservation of the epithelial barrier during allergic inflammation involves TSLP/TLR/NF-κB-mediated expression of claudin 7 in DCs and this consequently may lead to an important therapeutic option in allergic diseases.

The study by Lin et al. (2010) focused on Orai1, the pore-forming subunit of Ca^{2+}-release-activated Ca^{2+} (CRAC) channels (Gwack et al. 2007). As immune system cell activation, proliferation and degranulation depend on Orai1 and involve calcineurin-/NFAT-signalling to express cytokine genes and other genes critical for the immune response, Lin et al. (2010) investigated Orai1 expression in normal and allergic rhinitis (AR) mice airway and spleen. Orai1 protein was found in mucosal epithelium and submucosal glandular epithelium of airway, and in immune cells of spleen with higer expression in AR mice compared with wild-type mice. The results indicated that Orai1 is upregulated in the airway and spleen in allergic inflammation and may participate in the pathogenesis of AR. The authors suggest that intervention measures interfering with Orai1/CRAC channels may offer novel and effective prophylactic and/or therapeutic means to control AR symptons.

Fatty acids and metabolites thereof (which includes n-3 and n-6 polyunsaturated fatty acids—PUFAs) have been demonstrated to regulate aspects of immune functions (Pompos and Fritsche 2002). Therefore, Tokuda et al. (2010) looked on the expression of fatty acid-binding protein 7 (FABP7), which is distinguished by its strong affinity for n-3 PUFAs (Nasrollahzadeh et al. 2008) and FABP5, in mouse peripheral immune organs. The authors suggested a role for FABP7 in T cell homeostasis as its expression was restricted to fibroblastic reticular cells (FRCs) of T cell areas in lymph nodes and spleen and due to an increase of $CD4^+$ cells in mesenteric lymph nodes lacking FABP7. It is hypothesized, that FABP7 might possibly down regulate IL7 production (IL7 promotes $CD4^+$ T cell survival) due to changes in the n-3 PUFA content of the FRC membrane.

Development

A comparative immunolocalisation study was performed with type II collagen and the proteoglycans aggrecan and perlecan as target proteins using 12–14-week-old human foetal and postnatal (7–19 months) ovine joints (Smith et al. 2010). Perlecan, which has well-established roles in chondrogenesis (Iozzo 1994) and which is also a basement membrane component (Melrose et al. 2004) followed a virtually identical localisation pattern [(inter)territorial localisation] as that of type II collagen, the major structural collagen of cartilage and a chondrogenic marker beyond contention (Eyre et al. 2002, 2006; O'Driscoll et al. 1995). However, a slightly divergent localisation pattern in postnatal cartilages was observed where perlecan displayed a strict pericellular localisation pattern around the chondrocytes of permanent articular cartilages. Based on these findings, the authors consider perlecan to be a (cell associated) chondrogenic marker of pre- and postnatal cartilages.

In cells of the rat enamel organ and in odontoblasts, co-expression was recently reported for tumour surface protein EMMPRIN (an immunoglobulin superfamily member that induces extracellular matrix metalloproteinase and which is also named CD-147 or basigin) and MMPs (matrix metalloproteinases), which contribute to progression of malignancies and tumour invasion. This suggests EMMPRIN-regulated MMP expression in differentiating ondontoblasts and ameloblasts (Schwab et al. 2007). Xie et al. (2010) extended research on this topic and observed upregulated (highest level was seen at postnatal day 1) and temporospatial expression of EMMPRIN during development as well as arrest of tooth germ development and decreased MMPs expression, when EMMPRIN transcription was experimentally reduced. These results cleary show a vital role of EMMPRIN on epithelial–mesenchymal interactions during tooth germ morphogenesis.

Embryoid bodies (EB) recapitulate to a limited extent embryonic development and therefore represent useful tools to study the interplay of different germ layers and their influence on cell differentiation. Pekkanen-Mattila et al. (2010) explored expression of several germ layer markers in the early stages of EB differentiation using eight human embryonic stem cell (hESC) lines with different cardiac differentiation potentials. EB formation and growth as well as the temporal and spatial organisation of germ layers was found to occur similarly in all hESC lines during early EB differentiation. No correlation between the cardiac differentiation potential of hESC lines and early germ layer markers was detected.

Cytokeratines are found in most epithelial cells (K5/14 for basal/myoepithelial and K8/18 for luminal cells) and expression of specific cytokeratines associate with putative stem/progenitor cells [K6 for body cells of the terminal end bud and K8/14 for luminal progenitor cells—(Smith et al. 1990)]. As a comprehensive expression analysis of such cytokeratines during embryonic and early postnatal development has not been conducted, Sun et al. (2010) addressed this issue. They could demonstrate that aforementioned cells already exist in embryonic mammary glands. With

respect to K5 and K14 the authors found that only K14 was transiently expressed in the suprabasal/luminal compartment of the pre-pubertal mammary epithelium, indicating transient disconnection of K5 and K14 expression. With additional insights into usage of cytokeratines as mammary epithelial differentiation markers, such molecules may also be used in overexpression or ablation studies to specifically look into mammary development.

Maspin, a serine protease inhibitor (Zou et al. 1994) expressed in osteoblasts and promoting accumulation of latent TGF-β in bone ECM (Tokuyama et al. 2007), was the object of investigations by Davaadorj et al. (2010) on a role of this molecule in tooth development. The importance in this process was demonstrated when a neutralizing maspin antibody was employed, which led to inhibition of proper dental tissue formation in in vitro organ cultures of rat mandibular first molars as well as to growth suppression of cultured rat odontogenic epithelial and human dental papilla cells.

TGF-β1 (Worster et al. 2000) and collagen (Chen et al. 2005) or collagen hydrolyzate (Bello and Oesser 2006; Moskowitz 2000) are players in mesenchymal stem cell-mediated chondrogenic differentiation/articular cartilage repair and degeneration. Both molecules were used to analyse the applicability of adipose-derived stromal cells (ADSCs) in chondral defects (Raabe et al. 2010). TGF-β1 and hydrolyzed fish collagen were found to be very effective stimulants for differentiation of the chondrogenic lineage making ADSCs in equine veterinary tissue engineering useable in cartilage repair.

Reproduction systems

Expression of lung surfactant proteins (SPs) in human first-trimester placental tissues (22–56 days p.c.) was addressed by Sati et al. (2010), as extrapulmonal occurence of such molecules has been reported (Snyder et al. 2008; Madsen et al. 2003; Kankavi et al. 2007; Eliakim et al. 1989), inter alia in the female reproductive tract and amniotic fluid (Leth-Larsen et al. 2004; Miyamura et al. 1994). SPs were observed in the trophoblastic layers of chorionic villous tree, trophoblastic cell columns, Hofbauer cells, angiogenic cell cords, vascular endothelium and foetal haematopoietic cells. Due to the detection of SPs in such placental structures/cells it was speculated that SPs might have a role in placental immunobiology (considering the immunological paradox of the survival of the allogeneic conceptus), angiogenesis and vasculogenesis processes during early pregnancy or phagocytotic processes involving Hofbauer cells, which represent foetal placental antigen-presenting cells.

Lecce et al. (2010) investigated localization of CD43, a sialoglycoprotein with major glycoforms of 115 and 130 kDa (Fukuda and Tsuboi 1999; Remold-O'Donnell et al. 1986; Serrador et al. 1998), in uterine epithelium cells (UECs) during early pregnancy using tissues obtained from pregnant rats. UECs from ovariectomised rats treated with ovarian hormones were also used. The authors found in pregnant rats prior blastocyst implantation and in oestrogen-treated ovariectomised rats a 95-kDa form of CD43 localizing basally in UECs, but a apical 85-kDa form in UECs at the time of implantation and in UECs of ovariectomised rats under conditions of progesterone treatment. It is concluded that for facilitation of blastocyst attachment CD43 moves in a progesteron-dependent manner from the basal to the apical epithelium surface involving lower-molecular-weight forms of CD43, which more likely contributes to adhesion conditions rather than CD43 molecules with high glycosylation levels which have more de-adhesion properties (Fukuda and Tsuboi 1999; Remold-O'Donnell et al. 1986; Serrador et al. 1998).

Extracellular adenosine, produced by ecto-50-nucleotidase (CD73) and ectonucleoside triphosphate diphosphohydrolase (E-NTPDase) family members, plays fundamental roles in sperm motility and fertility (Fraser 2008; Minelli et al. 2004; Schuh et al. 2007). So far, expression analysis of CD73 along the male murine reproductive tract has not been conducted. Applying immunocytochemical techniques and in situ enzymatic assays, Martin-Satue et al. (2010) observed high CD73 expression along the mouse male reproductive tract (i.e. in germinal, somatic and Leydig cells, smooth muscle layers of the vas deferens and co-localization with NTPDase3 in secretory epithelia of accessory glands and epidydimis). The major conclusions drawn were that CD73 and NTPDase3 influence male fertility through control of extracellular nucleoside/nucleotide levels in spermatogenesis, control of smooth muscle contraction, and influence of sperm fluid composition.

Studying the insulin pathway, Sun et al. (2010) demonstrated that culturing 12.5 dpc fetal mouse ovaries with insulin had a profound detrimental effect on oogenesis and folliculogenesis in vitro leading to significant oocyte growth retardation and a reduced proportion of secondary follicles at 12.5 days post-coitum (dpc). Zhang et al. (2010b) investigated the effect of continuous insulin exposure on folliculogenesis in 16.5 dpc and 3-day post partum (dpp) mouse ovaries in vitro taking the Akt signalling pathway into account which is mainly involved in folliculogenesis (Liu 2006; Liu et al. 2006). The authors found that insulin-mediated regulation of folliculogenesis via Akt occurred stage-specifically, as insulin promoted Akt phosphorylation and the primordial follicular assembly and activation process in 16.5 dpc foetal mouse ovaries, whereas in cultured 3 dpp mouse ovaries, repression of Akt phosphorylation was observed.

Mouse vasa homolog (MVH) localization was systematically explored during mammalian spermatogenesis by Onohara et al. (2010). MVH, a DEAD-box family member of ATP-dependent RNA helicases, participates in several aspects of RNA metabolism and translational events (Raz 2000; Tsai-Morris et al. 2004) and is critically involved in germ cell development/male sterility (Tanaka et al. 2000). It localizes to intermitochondrial cement (IMC) and chromatoid bodies (CBs), two cytoplasmic compartments classified as *nuage* (Noce et al. 2001; Toyooka et al. 2000) and which represent various forms of dense material in germ cells of a wide variety of species (Eddy 1974, 1975). The authors identified fine particles (in diplotene and meiotic cells), IMC (in pachytene spermatocytes after stage V), loose aggregate of strands (in late spermatocytes) and CBs (in round spermatids) as *nuage* compartments and observed a clear discontinuity of *nuage* transition between spermatocytes and spermatids.

Fuc-glycoconjugates have been shown to be important in mammalian spermatogenesis (Martinez-Menarguez et al. 1993; Morokuma et al. 2007; Tang et al. 1982). That is why it was quite surprising that glycoconjugate research has not been conducted so far in *Xenopus laevis* (an extensively employed animal model). Using four different Fuc-binding lectins, proteins containing defined carbohydrate-recognition sites, Valbuena et al. (2010) addressed this shortfall, characterizing distribution of such molecules in *Xenopus* testis. With labelling of spermatocytes and spermatids by the orange peel (*Aleuria aurantia*) lectin and the asparagus pea (*Lotus tetragonolobus*) agglutinin and the finding through deglycosylation pretreatments that such fucosylated glycans were of N- and O-linked types, first steps towards gaining informations regarding the nature and involvement of glycoconjugates in testicular function have been made.

A role of the "endocannabinoid" system [i.e. anandamide (AEA); AEA receptors—cannabinoid receptors (CB) 1 and 2 and AEA modulating enzymes—fatty acid amide hydrolase (FAAH) and N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD)] in implantation and early pregnancy maintenance has previously been suggested. Extended work on this topic was provided by Taylor et al. (2010) who investigated involvement of cannabinoids during endometrial transformation, using well-characterised human menstrual cycle biopsy samples. Main elements (receptors and modulating enzymes) were found to coexist in many endometrial cell types and to be differentially expressed (FAAH and NAPE-PLD), assigning such enzymes a key role in control of local AEA levels during the menstrual cycle. Loss of CB2 receptor immunoreactivity between the menstrual cycle further suggested CB2 to be intimately involved in menstrual events.

Ectoplacental cone (EPC) -derived cells were the subject of studies conducted by Tesser et al. (2010), who focused on mouse trophoblast glycogen cells (GCs) in the context of blood cells and trophoblast giant cells (TGCs) during placentation, processing E5.5–E12.5 implantation sites. GCs were not observed throughout the EPC, rather originating in the upper portion in the midregion of EPC at 6.5, contained glycogen deposits at E6.5–E8.5 and were in close proximity to TGCs and maternal blood cells at E7.5–E8.5. Glycogen-derived glucose was suggested to drive invasion of GCs and TGCs during the process of placentation.

Methodical advances

BODIPY 493/503 is a lipophilic fluorescent dye, which has been used quite extensively to label lipid droplets (LDs). Depending on the sequence of exitations in double fluorescence labelling experiments, erroneous interpretations in the identification of LDs may occur. Ohsaki et al. (2010a) have addressed this problem by elaborating a protocol to preclude such a scenario.

Meunier et al. (2010) invented a new time-consuming, muscle fibre typing protocol applying a semi-automated histological approach to evaluate the percentage of different fibre types and their morphological parameters in histological sections. By dual labelling of serial cross sections of bovine muscles using antibodies against myosin heavy chain isoforms (for fibre classification) and laminin which allows surrounding fibre to be delineated precisely—(Miller and Stauber 1994), the authors found the results to be comparable to those obtained by electrophoresis-based fibre characterisations (Rivero et al. 1997). Additional advantages were that toxic solutions were avoided (such as the frequently used diaminobenzidine-tetrahydrochloride) and the ability of the method to also identify hybrid fibres. Morevover, the approach not only allowed muscle plasticities to be studied, but may also be applied to species-related typification purposes.

Witter et al. (2010) used a porcine experimental model of infrarenal aorta dissection to examine the effect of different tissue glues on fibre composition, vascular smooth muscle cell (VSMC) phenotype, free vessel lumen and the cells of the immune system 12 months postoperative survival. The tissue glues applied contained either glutaraldehyde or formaldehyde as protein cross-linking molecules or were based on fibrin. All types of glue caused stenosis or had an impact on the immunohistochemical phenotype of VSMC and the number and distribution of vasa vasorum in the aortic wall. Although some tissue glues caused more severe histopathological changes than others, the authors concluded that beside the adverse effects, additional parameters, such as glue handling and adhesive properties, need to be taken into account for the choice of the most appropriate glue.

Lamellar bodies in type II alveolar epithelial cells were addressed by Vanhecke et al. (2010). In ultrathin sections of chemically fixed and epoxy resin-embedded cells they appear as osmiophilic vesicles with tightly packed, more or less periodically arranged lamellae (Douglas et al. 1975). However, the periodicity of lamellae depended on the chemicals used (Stratton 1977). Alternative methods (cryofixation, cyrofixation by freeze substitution, freeze fracturing, cryo-electron microscopy of vitreous sections—CEMOVIS (Al-Amoudi et al. 2004; Dubochet et al. 1988)] were in fact less prone to artefacts but made studies on lamellar bodies still difficult. Vanhecke et al. (2010) further developed the high-pressure cryofixation approach, in combination with CEMOVIS and the use of perfluorocarbon to avoid alveolar collapse prior to fixation by high-pressure freezing. Without the use of any chemical fixatives, cryoprotectants, dehydration solvents or staining procedures, ice crystal-free samples of bulk lung tissue up to 200 μm thickness containing alveolar epithelial type II cells were obtained and enabled the authors to examine the native lamellar body ultrastructure under near in vivo conditions. The approach opened up the possibility to scrutinize various theories of lamellar body biogenesis, exocytosis and recycling.

Scanning probe microscopy (SPM) can visualize structure and mechanics of biological components as well as quantify adhesive forces between molecules and cells or reveal the viscoelasticity of cells or tissues by force measurements. It has also been applied to determine dynamical behaviours of biological components. Conventional contact-mode SPM have a time resolution in the range of minutes; therefore, fast dynamics in living cells cannot be monitored. High-speed SP microscopes achieve a temporal resolution of <0.1 s. Unfortunately, high-speed SPM applies large frictional and compressive forces on samples which would compromise the integrity of living cells. Tamura et al. (2010) combined soft cantilever to modulate the force applied to cells, low feedback-gain operations of a commercial SP microscope, and imaging of cantilever deflection. Using this improved combination the authors were able to achieve live-cell imaging of the mechanical architecture in leading lamellae with a temporal resolution of about 10 s.

The project handled by Stollenwerk et al. (2010) delved into the development of standard protocols for synthesis of gram amounts of well-characterised nanoparticles for use in molecular magnetic resonance imaging (MRI). This aim was initially tackled by identifying critical control points allowing fine-tuning of the method to produce exactly specified nanoparticles of a pre-defined size. The authors prepared gadolinium-bearing (to allow detection of the particles by MRI) albumin–polylactic acid nanoparticles in the size range 20–40 nm diameter. Challenges to meet included the warranty of chemical purity and to minimize exposure of animals or humans to nanoparticle-generating gadolinium, which is highly toxic in non-chelated form. As size may determine the outcome of interactions within the tissue and due to the immense surface area of nanoparticles, standardization of the nanoparticle size and thus surface was of utmost importance. The outcome of this study revealed that standardising the synthesis protocol did not lead to a standardised product (e.g. nanoparticle sizes displayed a significant intrabatch and interbatch variability and were micellar prior to lyophilisation, but loosely structured aggregated masses after lyophilisation and subsequent resuspension). However, such nanoparticles paved the way for further developments. In series II, analyses revealed that dialysis failed to remove educts, which skewed size distributions. Therefore, major alterations, conducted by Abdelmoez et al. (2010), included replacement of dialysis with diafiltration and to de-fat and stabilise the human serum albumin (HSA) prior to preparing HSA-DTPA conjugates. This resulted in HAS molecules homogenous in size. The inclusion of further testing methods allowed standardised particle sizes to be achieved and accurate measurement of critical physicochemical parameters influencing particle size and imaging properties. However, the authors regarded the HSA-based nanoparticles not yet suitable for upscaling into industrial production, but excellent reagents for exploratory work. Future studies will therefore focus on MR imaging, biodistribution and pharmacokinetics of these particles in animal models.

Sobol et al. (2010) aimed to optimize the procedure of cryoimmobilization of suspension cells for electron microscopy. The authors therefore examined various conditions of freeze-substitution procedures and determined conditions that allowed high reproducibility and most importantly good preservation in particular nuclei and nucleoli (considering the fact, that such intracellular target sites represent the most difficult structures with regard to effective verification and saturation with resin), in addition to antigens for immunogold labelling. The developed protocol was also less time-consuming than standard protocols.

The use of silver and silver compounds, widely applied as a remedy for a variety of different illnesses, has to be revisited considering studies by Danscher and Locht (2010). They could prove, applying autometallography, in vivo liberation of electrically charged silver atoms/silver ions from metallic silver surfaces (i.e. metallic silver pellets, silver grids and silver threads) within the brain, skin and abdominal cavity, through a process referred to as dissolucytosis (Larsen et al. 2007). Bio-released silver ions were taken up by dissolucytotic macrophages, local cells of all kinds and ended up through passing into the lymph and blood streams in tissues all over the body. The blood–brain-barrier was also bypassed. Taking into account the

damaging effects of dissolucytotic released silver ions from metallic silver surfaces on many organs, it is now clear that implantation of silver or silver-plated devices are not recommendable anymore.

Reviews

That actin is a karyophilic key protein required for many nuclear processes (Chen and Shen 2007; Schleicher and Jockusch 2008) has been discussed controversially quite a long time. But during the past decades the relevance of actin in the nuclear compartment has finally been appreciated. Filamentous actin (F-actin) has not been found in the nucleus, explaining why nuclear actin is unable to bind phalloidin (Cooper 1987). There are also several well-known actin-binding proteins (ABPs) that are additionally found in the nucleus although many of them bind to F-actin only. Castano et al. (2010) reviewed the current knowledge of nuclear ABPs and provided the reader with a wealth of informations regarding ABPs and their link to processes related to chromatin remodelling and their impact on key nuclear events, such as transcription initiation and elongation, DNA repair and DNA replication. The authors outlined, that a pool of nuclear complexes involved in chromatin remodelling may interchange factors and that nuclear actin may function as a key protein for interchanging such factors by bringing them together. In other words, actin and associated proteins may help to create a dynamic scaffold in which nuclear processes can take place. Through the association of actin with the nuclear envelope-associated proteins emerin and lamin A, these molecules have *non-classical* ABP function The authors also pointed to additional roles of these ABPs in nuclear architecture and dynamics (i.e. positioning and movement of the nucleus within the cell).

Weigert et al. (2010) provided an overview nf potential applications of intravital microscopy (IVM), with a particular emphasis on subcellular imaging. The authors focused on optical microscopy techniques to perform kinetic and functional studies in living animals, primarily small rodents. The use of intrinsic or endogenous fluorescent molecules in living animals can be a powerful tool for basic research or diagnostic purposes, e.g. molecules such as NAD(P)H were measured and visualized in living mice during microcirculatory failure, ischaemia and reperfusion in the liver (Guan et al. 2009; Paxian et al. 2004), or in the kidney in response to LPS-induced sepsis (Wu et al. 2007). Collagen, elastin, myosin and myelin fibres or lipid-enriched structures (Evans et al. 2005; Fu et al. 2008; Konig et al. 2007; Llewellyn et al. 2008) have been imaged in living animals to investigate structural aspects. Tissue architecture and function can be also imaged by introducing genetically engineered fluorescent proteins or administering exogenous dyes. IVM also opend new insights into many fields such as cancer biology, immunology, microbiology and recently in stem cell research by determining the fate of a single cell. Even higher resolution has been employed to visualize processes at subcellulars levels. An obstacle that had to be overcome were motion artefacts due to respiration and heartbeat. For that purpose surgical procedures and novel devices ensuring the stabilization of the organ have been employed. In conclusion, the authors described new developments in non-linear optical microscopy for in vivo studies which have addressed key questions in various aspects of tissue biology.

In their review Pol et al. (2010) focused on the three major desorption ionization techniques used in mass spectrometry imaging (MSI), the first technique that visualizes the distribution of small molecules in tissues (Debois et al. 2009). After describing the technical principles, the authors concentrated on the practicability of desorption ionization of secondary ion mass spectrometry (SIMS), matrix-assisted laser desorption ionization (MALDI) and desorption electrospray ionization (DESI) in biomedical and life science imaging applications. Making it difficult to summerize such a wealth of information with respect to the multiple applications for SIMS, MALDI or DESI, a key feature of biological MSI is that it enables the user to image molecules that are hard to visualize by other imaging techniques. Thus MSI represents an additional important approach to gain deeper insights into diagnostic and therapeutic histological and cellular mechanism. Of further importance is the fact that unlike other imaging techniques, no labelling procedures are required. With respect to protein MSI, no sample treatments need to be performed except that the tissue has to be flash-frozen, sliced and mounted on appropriate surfaces.

Miscellaneous

Shin et al. (2010) reported on studies on tissue accumulation of daunorubicin (DR), doxorubicin (DX) and epirubicin (ER) post i.v. injection. Such cancer relevant antibiotics display, despite structural similarities, large variations in pharmacokinetic parameters. Applying immunocytochemistry to rat kidney tissue with an antibody reacting with theses drugs the authors found differences in the detection of the molecules 120-h post injection, with only DR not being detectable. Similar observations were made in the pancreas, hair follicle and stomach (Ohara et al. 2007a, b; Shin et al. 2009), indicating a stronger propensity of cellular accumulation for DX and ER in such tissues. The authors concluded that the knowledge gained from their investigation may help to develop a better understanding of the individual

properties of DR, DX and ER with respect to the pharmacokinetic parameters and anti-tumour activities.

The observation that stromal fibroblasts derived from basal cell carcinoma or squamous cell carcinoma influence the phenotype of normal human keratinocytes (in terms of intermediate filament expression) and the fact that such changes occur when stromal fibroblasts and normal epithelium were physically separated (Lacina et al. 2007a, b) (indicating an involvement of stromal cell-produced soluble molecules on the functional phenotype of keratinocytes) prompted Strnad et al. (2010) to compare the transcriptomas of normal fibroblasts and squamous cell carcinoma-associated fibroblasts applying microchip array technology. The authors found 560 differentially expressed genes. Two growth factors, IGF-2 and BMP-4 were determined as candidate factors responsible for tumour-associated fibroblast activity influencing differentiation of normal epithelia. The authors consider these molecules and/or their receptors as potential therapeutic targets.

Due to differences seen in labelling of musculars satellite cells (SCs) with the marker protein neural cell adhesion molecule (NCAM) and Pax7, Lindstrom et al. (2010) applied immunohistochemistry to trapezius muscle biopsies from power lifters and sedentary men, assuming that SCs are intrinsically different or display different stages of quiescence, activation or differentiation. Using markers for SC activation and differentiation (MyoD, myogenin, c-Met and Dlk1) the authors extended their studies in conjunction with the reference markers NCAM and Pax7. They found SCs to show pronounced heterogeneity in activation, myonuclei turnover, muscle fibre growth and muscle fibre damage and repair. The results thus demonstrated that human SCs are more heterogeneous than previously recognized.

OCT4 is an embryonal transcription factor and is considered to be an important diagnostic tool for germ cell tumours (GCT). In this regard, Mueller et al. (2006) recently reported resistance to cytotoxic (i.e. cisplatin) treatment and lack of OCT4 expression in embryonal carcinoma (EC) cells, which are considered to be the malignant counterparts of embryonic stem (ES) cells. Mueller et al. (2010) hypothesized that such a cell type exists in nonseminomatous GCT (NSGCT—a subtype of testicular GCT), and may drive the growth of cisplatin refractory tumours. Employing an NSGCT xenograft model derived from the cell lines H12.1 and 1411HP which gave rise to OCT4-positive, cisplatin-sensitive and cisplatin-resistant, OCT4-negative xenografts, respectively, in conjunction with patient-derived NSGCT samples, the authors suggested that malignant growth of cisplatin-resistant NSGCT may be driven by Oct4-negative EC cells.

Growth and migration pattern of pterygial cells in the pathogenesis of pterygium, a benign growth of the conjunctiva on the corneal surface, were characterized by Bai et al. (2010). Particular focus was made on the spatial expression pattern of markers for stem cells, cell growth, and matrix metalloproteinases. The authors found a spatial existence of stem cell-like cells in the head (on the cornea) and body (on the conjunctiva) epithelia of pterygial tissues, but not in the neck (over the focal limbus) region, consistent with the observation of the body region having the highest proliferative aptitude. It is concluded that the pterygium neck is the proliferative gear for pterygium growth. Pharmacological intervention targeting matrix metalloproteinases or molecular intervention by gene silencing to reduce proliferative cell activity might therefore be effective instruments to impede pterygium recurrence.

The observation that exposure of human acute myeloblastic leukaemia (HL60) cells to monocytic and granulocytic differentiation inducers resulted in Akt and PKC phosphorylation during the late stage of differentiation and increased expression of mammalian target of rapamycin (mTOR), a downstream signalling protein of Akt (Yamada et al. 2008, 2010) provoked Yamada et al. (2010) to focus on mTOR-related signalling proteins (i.e. Raptor of mTORC1 and Rictor of mTORC2) in differentiating HL60 cells. Applying siRNA technology and inhibitors of PI3K/Akt and PKC pathways, activation of Akt and PKC was seen to be related to certain cell functions, normally detected in differentiated cells, with Rictor being an upstream regulator in this event. The findings were supported by previous observations, showing phosphorylation of Akt by Rictor (Sarbassov et al. 2005) and PKCs by TORC2 (Guertin et al. 2006; Jacinto et al. 2004).

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