Immunodetection of lipid droplets by targeting adipophilin (PLIN2) in tissues of rats, mice, dogs, minipigs and non-human primates

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Context

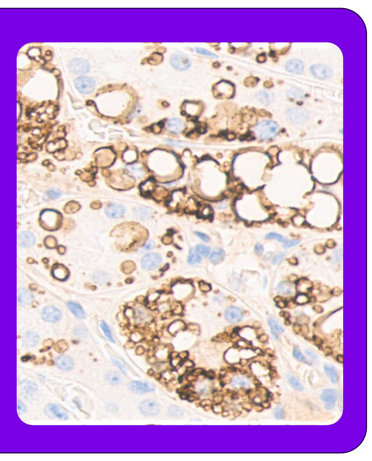
Drug-induced cytoplasmic vacuolation is commonly observed in preclinical toxicological studies and can be of various nature (*eg.* phospholipidosis, fatty change, increased autophagy). Differentiating non-phosphorylated lipid accumulation from phospholipidosis can be particularly challenging on standard H&E sections. Histochemical fat-staining methods such as Sudan Black and Oil red O are useful but lack sensitivity and require frozen tissues, while transmission electron microscopy which is the gold standard for confirming phospholipidosis, is of low-throughput and not readily accessible. Immunohistochemistry (IHC) has been proven to be a simple, quick and sensitive method for reliably differentiating lipidosis and phospholipidosis in FFPE livers from rats, by targeting adipophilin (non-lysosomal lipid droplets-associated protein) and LAMP-2 (Lysosome-associated membrane protein 2) respectively [1]. A previous IHC protocol had been validated in rodents in our laboratory but the new commercially batch (Abcam ab52356) was not reacting anymore with rat adipophilin.

Objective

Develop and validate an IHC protocol against adipophilin, suitable in our routine formalin-fixed paraffin-embedded (FFPE) tissues from commonly used preclinical species: rats, mice, dogs, minipigs, and non-human primates (NHP) samples.

Adipophilin: a lipid droplet-specific marker

Also known as Perilipin-2 (PLIN2), adipophilin is a protein coating neutral lipid droplets (LD) involved in lipid metabolism, transport and storage. It is ubiquitously expressed and serves as a general surface marker of LDs in non-adipose tissues and a reliable indicator of LD accumulation under physiological and pathological conditions [2,3].



Typical PLIN2 staining, dog, kidney x40
brown, intracytoplasmic, surrounding lipid droplets

MATERIAL & METHODS

Tissues: Adrenals, Skin, Liver, Kidneys

- Source: internal biobank - control groups from toxicological studies
- Preservation: FFPE blocks
- Species: Sprague-Dawley rats, CD1 mice, beagle dogs, Göttingen minipigs, cynomolgus monkeys

IHC technique

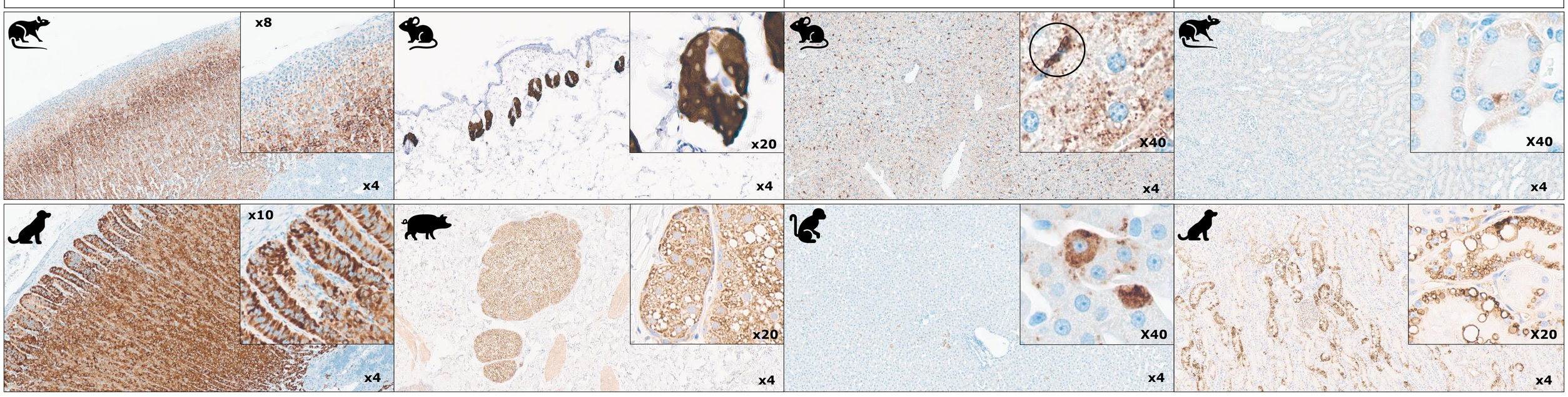
- Ventana Discovery Ultra instrument (Roche)
- Antigen retrieval: CC1 (40 min, 95°C)
- Amplification-Detection: OmniMap HRP (12 min)-Discovery ChromoMap DAB kit (Ventana)
- For each sample: negative controls obtained by replacing the primary antibody with SignalStain® Antibody Diluent (Cell Signaling Technology)

Validated IHC protocols

Species	Primary Antibody	Concentration (dilution)	Incubation conditions	Blocking (before and after primary antibody)
Rat, Mouse	Novus NB110-40877, Rabbit polyclonal Ab	10 µg/ml (1/100)	1h, 37°C	none
Monkey	Cell Signaling 95109, Rabbit monoclonal Ab			
Minipig	Abcam ab108323, Rabbit monoclonal Ab			Protein Block Serum-Free (2x4min; Cat. X0909, Dako)
Dog	OriGene ABIN112185, Mouse monoclonal Ab	0,5 µg/ml (1/100)	2h, RT	none

RESULTS

ADRENALS	SKIN	LIVER	KIDNEY
ALL SPECIES: strong signal in **adrenal cortex** with variable zonal distribution ▪ RODENTS: mostly *zona fasciculata* with only mild staining of *glomerulosa* and *reticularis* ▪ DOG: diffusely strong in all three layers of cortex ▪ MINIPIG: strong in *zona fasciculata* and *glomerulosa*, and mild in *reticularis* ▪ MONKEY: strong in *zona fasciculata*, moderate in *glomerulosa* and mild in *reticularis* **Medulla**: only rare positive cells (most likely remnants of adrenocortical cells [4])	ALL SPECIES: prominent signal of **sebaceous glands**, surrounding lipid droplets with variable intensity between cells in the same gland ▪ MOUSE: strong obscuring the cytoplasm - marked loss of tissue architecture and detachment artifacts, probably secondary to antigen retrieval method, without impact on the signal ▪ RAT: marked ▪ DOG, MONKEY: moderate ▪ MINIPIG: moderate - note also marked specific staining of some striated myocytes, also described in humans [5] and marked staining of sweat glands	**Hepatocytes** ▪ RODENTS: rare moderate signal surrounding cytoplasmic lipid droplets, primary along cell periphery ▪ MONKEY, DOG, MINIPIG: only rare lipid droplets **Hepatic stellate cells** (Ito cells, circled below) ▪ RODENTS: lipid storage vacuoles markedly in mouse and moderately in rat ▪ MONKEY, DOG, MINIPIG: no staining - reported to be inconspicuous and fewer in number in healthy dogs [4]	**Tubular epithelial cells** ▪ DOG: marked, multifocal variably sized vacuoles mainly in proximal tubules, with lipid droplets located at basal pole ▪ MONKEY: moderate, multifocal ▪ MINIPIG: moderate, diffuse ▪ RODENTS: only rare positive vacuoles



CONCLUSION

We successfully validated **IHC protocols for the five species** mostly used in preclinical studies and found a reliable positive control for **adipophilin detection.**

Limited background was seen, mainly in capillary endothelium, hepatocytes (minimal, diffuse) and in plasma, red blood cells, nephrocytes (minimal), also observed in negative controls. A staining (moderate) was observed for mast cells in rats, confirmed with Toluidine blue staining, also observed in negative controls. This non-specific staining does not compromise lipid droplets interpretation.

Adrenal glands and skin were used as positive controls for adipophilin immunostaining, showing consistent signal in the *zona fasciculata* and sebaceous glands, respectively. The **adrenal gland was preferred as a positive control**. If unavailable, skin could be used cautiously in mice due to potential architectural alterations and detachment artifacts from IHC processing. In mice, the liver could also serve as a positive control due to strong staining in hepatic stellate cells.

References

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4. Pignatelli *et al*. Endocr Res. 1995 Feb-May;21(1-2):129-36.
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6. Bergman JR. Vet Pathol. 1985 Sep;22(5):427-38.

Abbreviations

Ab: Antibody
DAB: 3,3'-diaminobenzidine
FFPE: Formalin-Fixed Paraffin-Embedded
HRP: Horse Radish Peroxidase multimer
IHC: Immunohistochemistry
LD: Lipid Droplets
RT: Room Temperature

Disclosure: This work was conducted using tissue samples sourced from an internal biobank, all obtained in compliance with applicable regulations and animal ethics standards.
L. Sautier, E. Fisher, S. Pocholle, F. Verlaguet, G. Do-Nascimento and B. Gauthier are employees of Sanofi and may hold shares in the company.