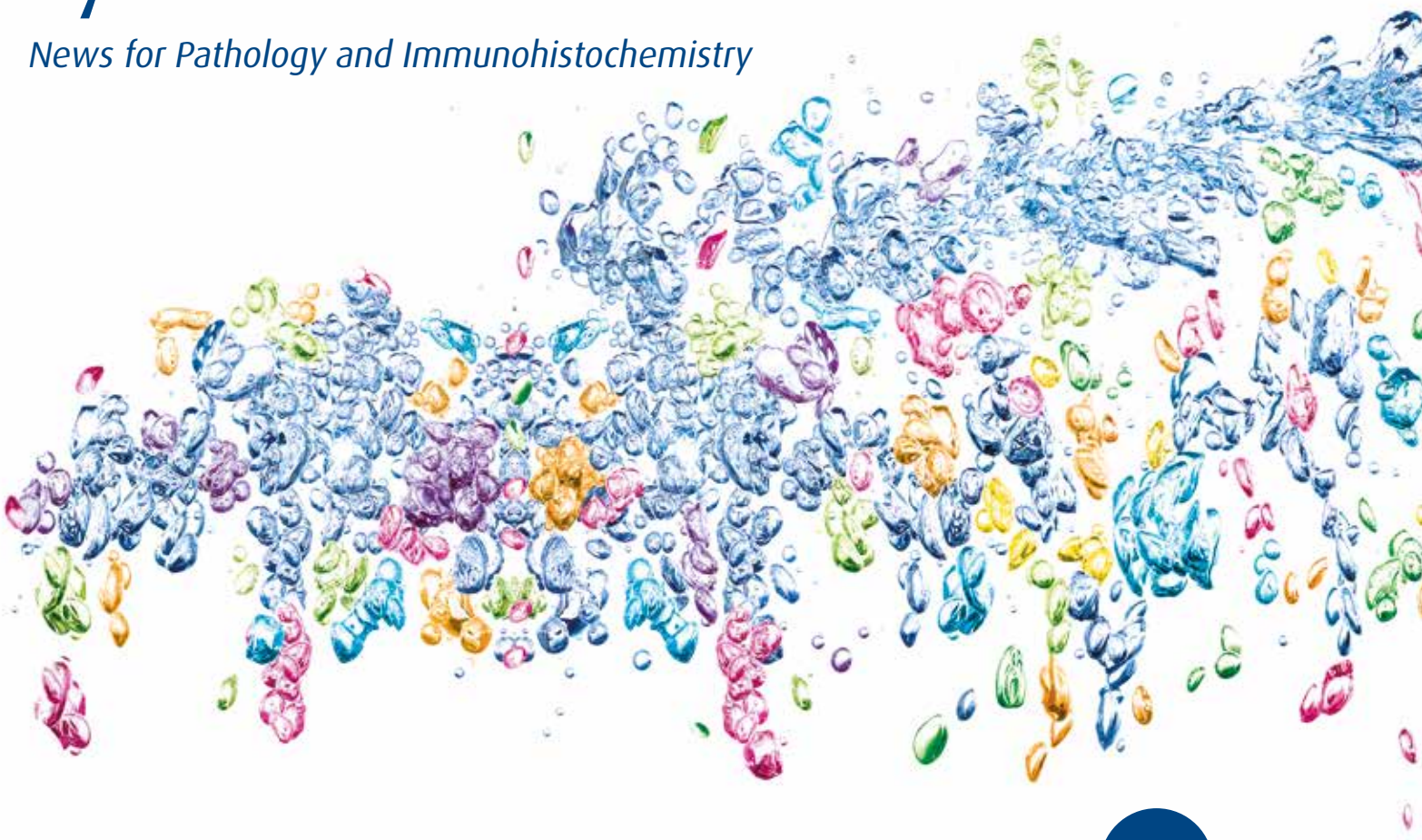
Zyto-Facts 1-2017

News for Pathology and Immunohistochemistry



Editorial

Dr. Karl-Georg Lintermann
PhD, Export manager

Zytomed Systems starts the new year with the launch of SMAD4 (DPC4), a prognostic marker for pancreas, colon and breast adenocarcinomas. Mutations in the SMAD4 gene lead to loss of expression and correlate with poor prognoses. Liver diagnostics and urothelial cancer often provide a challenge for pathologists who have to rely on immunohistochemistry in difficult cases for proper diagnosis. In this issue we discuss established and outdated antibodies, as well as newcomers for this purpose.
The „Focus: lab work" section addresses the topic of protein block in immunohistochemistry. You will find answers to questions as to which is the best block and why, as well as what the advantages of using high quality dilution buffers are. If you are interested in automated in situ-hybridisation, you would be well advised to consider our ZytoBrite Hybridizer TDH-500, which is now available internationally. This device has smart features like an USB port for data export, touch screen and heated water tank making associating consumables like humidity stripes unnecessary.

Enjoy reading!
Karl-Georg Lintermann

Events and Congresses

- **Frühjahrstagung 2017 der ÖGPath – IAP Austria**
  23–25 February 2017
  Tech Gate Vienna, Donau City, Austria
- **AMP Global 2017 Global Congress on Molecular Pathology**
  3–5 April 2017
  Estrel-Congress & Exhibition Center, Berlin, Germany
- **40e Assises de Pathologie 2017**
  18–19 May 2017
  Espace Tête d'Or, Centre de Congrès, Lyon, France
- **Histologica**
  9–10 June 2017
  Congress Centrum Oberhausen, Oberhausen, Germany
- **30e Congrès de l'Association Française d'Histotechnologie 2017**
  22–23 June 2017
  Nantes, ONIRIS, France
- **Congress of the German Society of Pathology 2017**
  22–26 June 2017
  Heinrich-Lades-Halle Erlangen, Germany
- **Medica 2017**
  13–16 November 2017
  Messe Düsseldorf, Germany
- **Carrefour Pathologie 2017**
  20–23 November 2017
  Palais des congrès, Paris, France

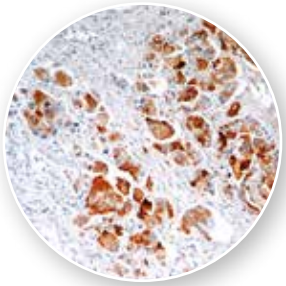
Uroplakins and Urothelial Origin

Uroplakin II: A highly specific and sensitive urothelial marker

Uroplakins Ia, Ib, II and III are structural proteins of terminal differentiated urothelial cells. In normal urothelia they are expressed in the luminal cytoplasmic membrane of umbrella cells. Immunohistochemistry with anti-Uroplakin III antibody (clone AU1) is widely used to identify urothelial origins. However, being highly specific, clone AU1 shows only a moderate sensitivity.

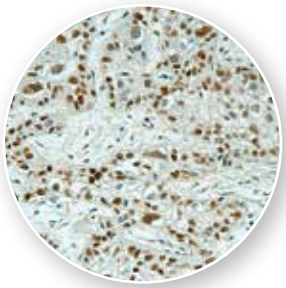
Uroplakin II, clone BC21 was first described in 2013 [1] as an urothelial marker with enhanced sensitivity for urothelial carcinoma. 44 out of 56 (78 %) urothelial tumours, including metastasis, were positive with Uroplakin II, whereas only 34 % exhibited positive staining with Uroplakin III (clone AU1). Further studies confirmed the excellent specificity and superior sensitivity of Uroplakin II [2, 3]. Smith *et al.* could not detect Uroplakin II staining in non-urothelial tissue. Li *et al.* concluded from their studies: *„UPII is a more valuable marker than UPIII in immunohistochemical analysis for confirming the urothelial origin of carcinomas."*

In a recent study Hoang *et al.* recommend a combination of Uroplakin II, GATA3 and p40 for the differential diagnostics of invasive urothelial carcinomas. These antibodies showed a high sensitivity of 92,2 % (71/77) even in less differentiated (grade 2 and 3) tumours.



Uroplakin II on urothelial carcinoma

GATA3 on urothelial carcinoma



GATA3 on urothelial carcinoma

Product information

Description	CE/IVD	Pre-treatment	Dilution	Volume	Cat No
Uroplakin II Clone: BC21 Host: Mouse	✓	HIER in Citrate pH 6.0	Ready-to-use	6 ml	MSG102
			1:50 – 1:100	0.5 ml	MSK102-05
Uroplakin III Clone: AU1 Host: Mouse	-	HIER in Citrate pH 6.0	1:25 – 1:50	0.5 ml	MSK052-05
GATA3 Clone: L50-823 Host: Mouse	✓	HIER in Citrate pH 6.0	Ready-to-use	6 ml	MSG100
			1:100 – 1:200	0.5 ml	MSK100-05
p40 (ΔNp63) Clone: BC28 Host: Mouse	✓	HIER in Citrate pH 6.0 or T-EDTA pH 9.0	Ready-to-use	6 ml	MSG097
			1:50 – 1:100	0.5 ml	MSK097-05
				1 ml	MSK097

Bibliography

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[2] Smith SC *et al.* Uroplakin II outperforms Uroplakin III in diagnostically challenging settings. Histopathol 65:132-138, 2014

[3] Li W *et al.* Uroplakin II is a more sensitive immunohistochemical marker than Uroplakin III in urothelial carcinoma and its variants. Am J Clin Pathol 142:864-871, 2014

[4] Hoang LL *et al.* Uroplakin II (UPII), GATA3, and p40 are highly sensitive markers for the differential diagnosis of invasive urothelial carcinoma. Appl Immunohistochem Mol Morphol 23:711-716, 2015

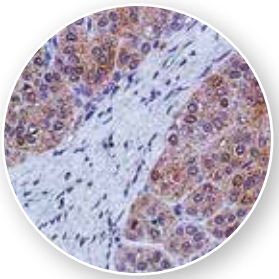
Arginase-1 and Glypican-3

The optimal combination for your immunostains on liver tissue

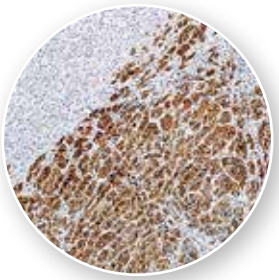
The distinction of hepatocellular carcinoma (HCC) from metastatic tumour in the liver as well as differential diagnostic of HCC vs benign lesions often present a diagnostic challenge. Recently, two antibodies, Arginase 1 and Glypican-3, proved to be very helpful in these diagnostic pitfalls.

Glypican-3 (GPC-3) is a heparan-sulfate cell surface oncofetal proteoglycan which is mainly expressed in hepatocellular carcinomas (HCC). In addition, GPC-3 expression can be found in hepatoblastoma, choriocarcinoma, yolk sac tumours and Wilm's tumours. GPC-3 immunohistochemistry is useful for differentiation of HCC from non-malignant liver tissue and other primary and metastatic lesions. By now numerous publications have shown that Glypican-3 is overexpressed in most HCC whereas normal liver tissue and benign lesions such as dysplastic and cirrhotic nodules are mostly negative for GPC-3 [1, 2, 3]. GPC-3 staining is recommended by the AASLD (American Association for the Study of Liver Diseases) for „tissue that is not clearly HCC" [4]. The marker may also be useful in differentiation of yolk sac tumours (+) and choriocarcinomas (+) from seminomas (-).

Arginase-1 is a key enzyme of the urea cycle and expressed mainly in hepatocytes. Arginase-1 as a marker for HCC was presented in the USCAAP meeting in 2010 and published in the *Journal of Surgical Pathology* [5]. Of 193 hepatocellular carcinoma (HCC) 96 % stained positive for Arginase-1. Only 2 of 557 non hepatocellular tumours, one cholangiocarcinoma and one prostate carcinoma, showed Arginase-1 expression. Both stainings were weak and focal. Additional publications confirmed the superior sensitivity and specificity compared to the classical liver marker HepPar-1 especially in poorly differentiated tumours [6,7].



Arginase-1 staining on HCC



Glypican-3 staining on HCC

Product information

Description	CE/IVD	Pre-treatment	Dilution	Volume	Cat No
Arginase-1 Clone: polyclonal Host: Rabbit	-	HIER in Citrate pH 6.0	Ready-to-use	7 ml	501-19281
			1:100	0.5 ml	501-19282
				1 ml	501-19284
Glypican-3 Clone: 1G12 Host: Mouse	✓	HIER in Citrate pH 6.0	Ready-to-use	6 ml	MSG067
			1:100 - 1:500	0.5 ml	MSK068-05

Bibliography

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[4] Bruix J and Sherman M. AASLD Practice Guideline. Management of Hepatocellular Carcinoma: An Update. Hepatology 2010 (published online at www.aasld.org)

[5] Yan BC *et al.* Arginase-1: a new immunohistochemical marker of hepatocytes and hepatocellular neoplasms. Am J Surg Pathol. 34:1147-1154, 2010

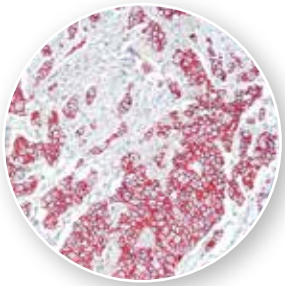
[6] Ordóñez NG. Arginase-1 is a novel immunohistochemical marker of hepatocellular differentiation. Adv Anat Pathol 21:285-290, 2014

[7] Fatima N *et al.* Arginase-1: a highly specific marker separating pancreatic adenocarcinoma from hepatocellular carcinoma. Acta Cytol 58:83-88, 2014

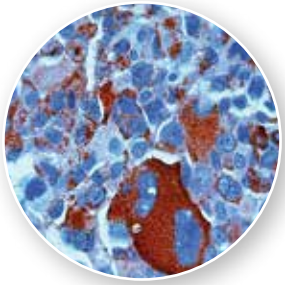
Protein Block in Immunohistochemistry

A protein block is mandatory to obtain an optimal signal to background noise ratio in immunohistochemistry.
Blocking usually takes place immediately before incubating the sample with the primary antibody. This blocking step reduces unspecific binding caused by electrostatic charge and hydrophobic interactions of the tissue with primary and secondary antibodies which can lead to strong background staining. Connective tissue, squamous epithelium and fatty tissue is frequently susceptible to this kind of background staining.

The classical approach for a protein block is to use normal serum from the same animal species in which the secondary antibody was raised. This is generally a goat serum because that is the species from which most of the secondary antibodies in commercial detection kits originate. Using a serum from the primary antibody source, for example a rabbit serum in combination with polyclonal antibodies, increases background through the binding of the secondary antibody to immunoglobulins of the blocking serum on the tissue.



E-Cadherin on mamma carcinoma: Concentrated antibody was diluted in Antibody Diluent (ZUC025) and stored for 24 month at 2-8 °C



HMB45 on malignant melanoma: Concentrated antibody was diluted in Antibody Diluent (ZUC025) and stored for 24 month at 2-8 °C

▶ Wash or decant the blocking solution?

For an efficient blocking decantation of the protein block is recommended. However, when using "sticky" blocking solutions like casein or skimmed milk powder, a proper wash is the method of choice. Otherwise over-blocking can mask the epitopes and the consequence is a reduced staining intensity.

▶ How to avoid an extra protein blocking step?

A separate protein blocking step can be omitted if the primary antibody is diluted in a high quality dilution buffer like Zytomed Systems' Antibody Diluent. These buffers contain reagents which effectively block unspecific binding and stabilize the primary antibody. Antibodies prepared from concentrates in Zytomed Systems' Antibody Diluent are usually stable for months.

Description	Amount	Cat No.
Antibody Diluent	100 ml	ZUC025-100
	500 ml	ZUC025-500

Suggested reading for the topic of hepatocellular carcinoma and immunohistochemistry

Nguyen T *et al.* Arch Pathol Lab Med 2015 Aug; 139:1028-1034

Comparison of 5 Immunohistochemical Markers of Hepatocellular Differentiation for the Diagnosis of Hepatocellular Carcinoma

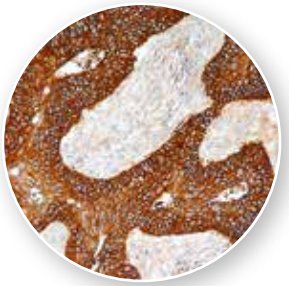
In this study antibodies against Arginase-1, HepPar1, CEA, Glypican-3 and BSEP (Bile Salt Export Pump Transporter) were presented and compared regarding their sensitivity and specificity for hepatocellular carcinoma. One focus of the study was the performance of the antibodies on dedifferentiated carcinoma. Arginase-1 and Glypican-3 proved to be the most stable markers as a combination of both enabled the identification of nearly all poorly differentiated HCC.

Open access to the full text under the following link: www.archivesofpathology.org/loi/arpa

EGFR Immunohistochemistry on Tissue of Varying Quality

Clone 2-1E1

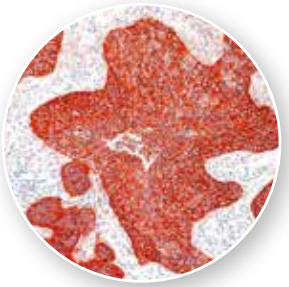
EGFR is highly overexpressed by a variety of human tumours such as breast and lung carcinomas, colon carcinoma and in tumours of the brain. In light of the new therapeutic options with small molecules and therapeutic antibodies, immunohistochemical detection of EGFR-overexpression has gained increasing importance over the last years.
The value of EGFR detection by immunohistochemistry is strongly influenced by the quality of the formalin-fixed tissue. When establishing a robust and reproducible immunostain it is of primary importance to use high quality EGFR antibodies.
One of the best commercially available EGFR antibodies is clone 2-1E1. After being discontinued for several years, Zytomed Systems now offers this clone again.
Immunohistostains using 2-1E1 show strong and specific signals even if the tissue is of suboptimal quality. The high quality of the antibody leads to a very good dilution factor making this antibody also an excellent choice in regard to cost-effectiveness.



EGFR, clone 2-1E1 immunohistochemistry using HRP-Polymer detection and DAB on squamous cell carcinoma of the lung

Product information

Description	CE/IVD	Pre-treatment	Dilution	Volume	Cat No
EGFR Clone: 2-1E1 Host: Mouse	-	Pepsin	1:100-1:200	0.5 ml	MSK014-05



EGFR, clone 2-1E1 immunohistochemistry using HRP-Polymer detection and AEC on squamous cell carcinoma of the lung

ZytoBrite TDH-500

Automated in situ-hybridisation

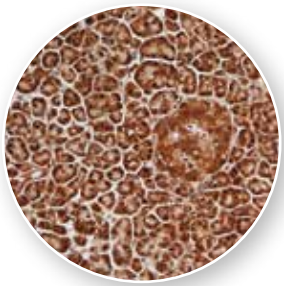
ZytoBrite TDH-500 Hybridizer is an easy to handle, cost effective device for automated denaturation and hybridization of FISH and CISH probes. The instrument is characterized by fast heating, high temperature accuracy and uniform temperature in the humid chamber. The data of each run can be recorded via USB port to ensure proper quality management of *in situ*-hybridizations. Up to 12 slides can be processed in one run.
The reliable ZytoBrite Hybridizer is used in numerous molecular pathology labs and is in daily operation in Zytomed Systems' quality control lab.

Advantages

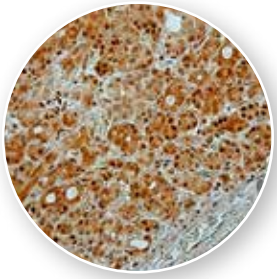
- Can be easily operated with the touchscreen or mouse
- 4 operation modes:
  1. Denaturation and hybridisation
  2. Hybridisation
  3. Custom program
  4. *In situ*-PCR
- Heated water tanks assure optimal humidification of the hybridization chamber
- Fast heating, high temperature accuracy
- Uniform temperature distribution in the hybridization chamber
- 60 program memory locations
- Data export function via USB port

SMAD4 (DCP4)

A prognostic marker for pancreas, colon, and breast adenocarcinomas



SMAD4 on pancreas



SMAD4 on pancreatic adenocarcinoma

Mothers against decapentaplegic homolog 4 (SMAD4) is a member of the SMAD family of signal transduction proteins. SMAD proteins are phosphorylated and activated by transmembrane serine-threonine receptor kinases in response to TGF-beta signaling. Activated SMAD proteins form homo- or heterodimers and then migrate into the nucleus, where they activate transcription.

SMAD4 is a tumour suppressor gene involved in growth inhibition. Mutation in the SMAD4 gene, which leads to loss of SMAD protein expression, was reported in 55 % of all Pancreatic Ductal Adenocarcinoma (PDAC). Other driver mutations in PDAC were described in KRAS, p53 and CDKN2[a].

Zapata *et al.* recommend a combination of SMAD4, Cytokeratin 19 and CA19-9 for the diagnosis of PDAC. Especially on small fine-needle aspirate samples in a metastatic setting this antibody panel has been found to be very helpful [1]. In numerous publications, loss of SMAD4 expression was reported to be an indicator of poor prognostic outcome in adenocarcinomas of the pancreas [1, 2, 3], colon [4], and breast [5, 6, 7].

Product information

Description	CE/IVD	Pre-treatment	Dilution	Volume	Cat No
SMAD4 (DCP4) Clone: SP306 Host: Rabbit	✓	HIER in Citrate pH 6.0	Ready-to-use	7 ml	519-6061
			1:100	0.1 ml	519-6060
				0.5 ml	519-6062
				1 ml	519-6064

Bibliography

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[2] Liu F. SMAD4/DPC4 and pancreatic cancer survival. Clin Cancer Res 7:3853-3856, 2001

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[5] Stuelten CH *et al.* Smad4-expression is decreased in breast cancer tissue: a retrospective study. BMC Cancer 6:25, 2006

[6] Liu N *et al.* SMAD4 is a potential prognostic marker in human breast carcinomas. Tumour Biol 35:641-650, 2014

[7] Liu N *et al.* SMAD4 expression in breast ductal carcinoma correlates with prognosis. Oncol Letters 10:1709-1715, 2015

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