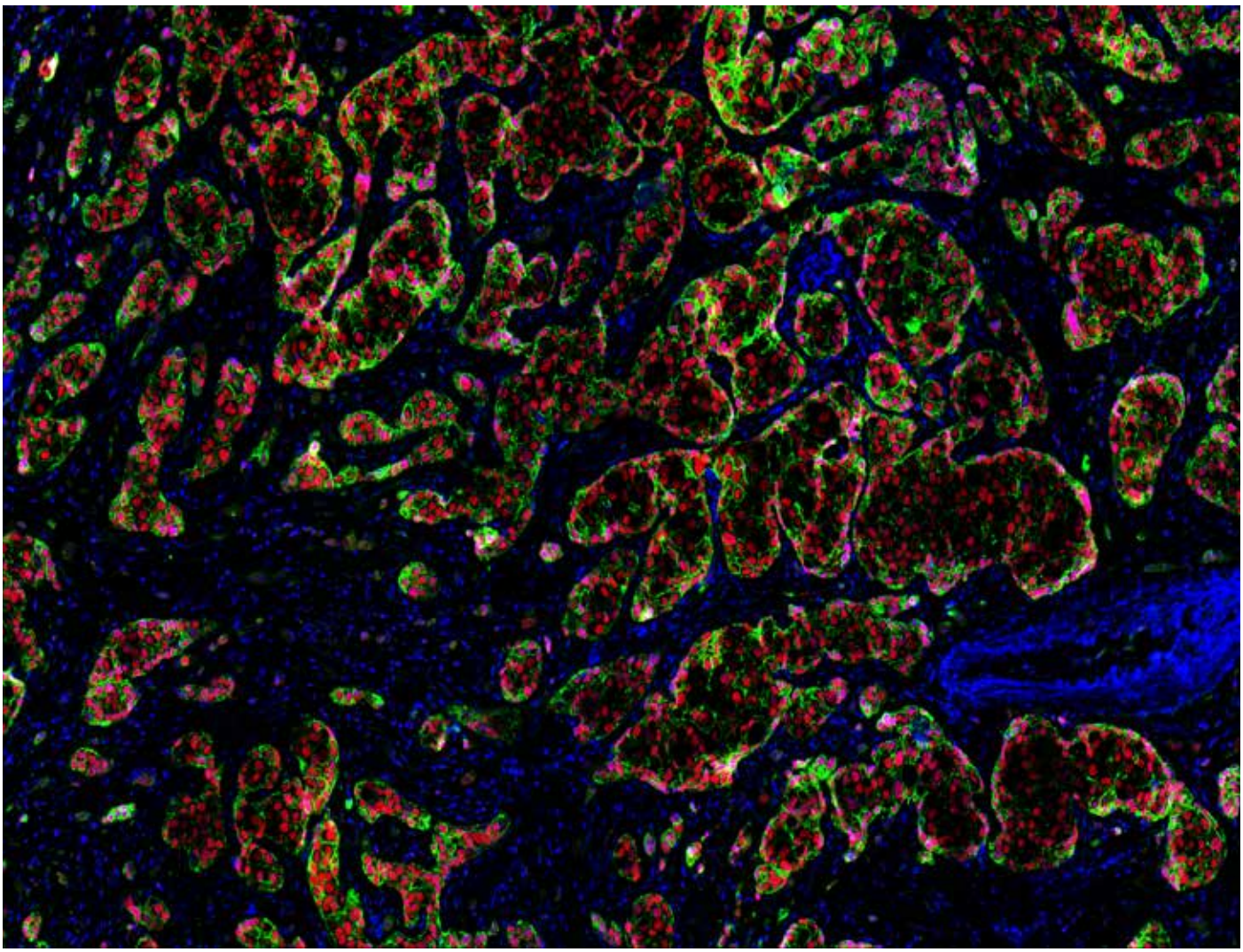

MECHANISMS IN CANCER

ATLAS ANTIBODIES

ATLAS ANTIBODIES

Mechanisms in Cancer

The past few decades have witnessed remarkable progress in understanding the molecular and cellular framework of cancer forming the basis for the novel innovative and efficient oncological therapies, during later years with a particular emphasis on harnessing anti-tumor immunity. As a result, several tumor markers are currently being used for a wide range of cellular processes in cancer.

Cancer is one of the world's most significant health problems, contributing to more than 9 million deaths yearly. The tissue of origin generally defines the tumor class. Carcinomas, which are epithelial malignancies, are the most frequent solid tumors. Squamous carcinomas arise from benign, precancerous lesions of the epidermis and non-secretory epithelia. Corresponding malignancies derived from hyperplastic glandular epithelia (adenomas) are called adenocarcinomas.

Tumors originating from the non-hematopoietic mesodermal lineage, e.g., connective tissue, skeleton, muscles, are collectively called sarcomas. Leukemias and lymphomas arise from the blood-forming (hematopoietic) cells in the bone marrow and from cells of the immune system, respectively. Finally, gliomas represent the most frequent types of malignant tumors of the central nervous system.

The tumor cells of different cancer types share several common properties and are characterized by their dedifferentiated state. These properties typically include sustained proliferation (immortality), escape from programmed cell death, genomic instability, as well as enhanced ability to move away from the sites of origin and the ability to invade other tissues in the body. The carcinogenesis can often be triggered by genetic mutations within the regulatory regions or reading frames of genes resulting in altered translation products or non-coding RNAs. In addition to this, the epigenetic regulation of gene activity may also induce cancer.

In this white paper, we present our highly validated primary antibodies for use as markers in cancer research.

PrecisA Monoclonals™ and Triple A Polyclonals™ are in-house developed antibodies for dedicated targets. We select clones recognizing unique non-overlapping epitopes and/or isotypes. By using our stringent production process and characterization procedure, our antibodies have a defined specificity, secured continuity and stable supply, overall offering premium performance in approved applications. They also permit high working dilutions, multiplexing opportunities and contribute to standardized assay procedures. The antibodies are commonly validated for use in immunohistochemistry (IHC), western blot (WB) and immunofluorescence (ICC-IF).

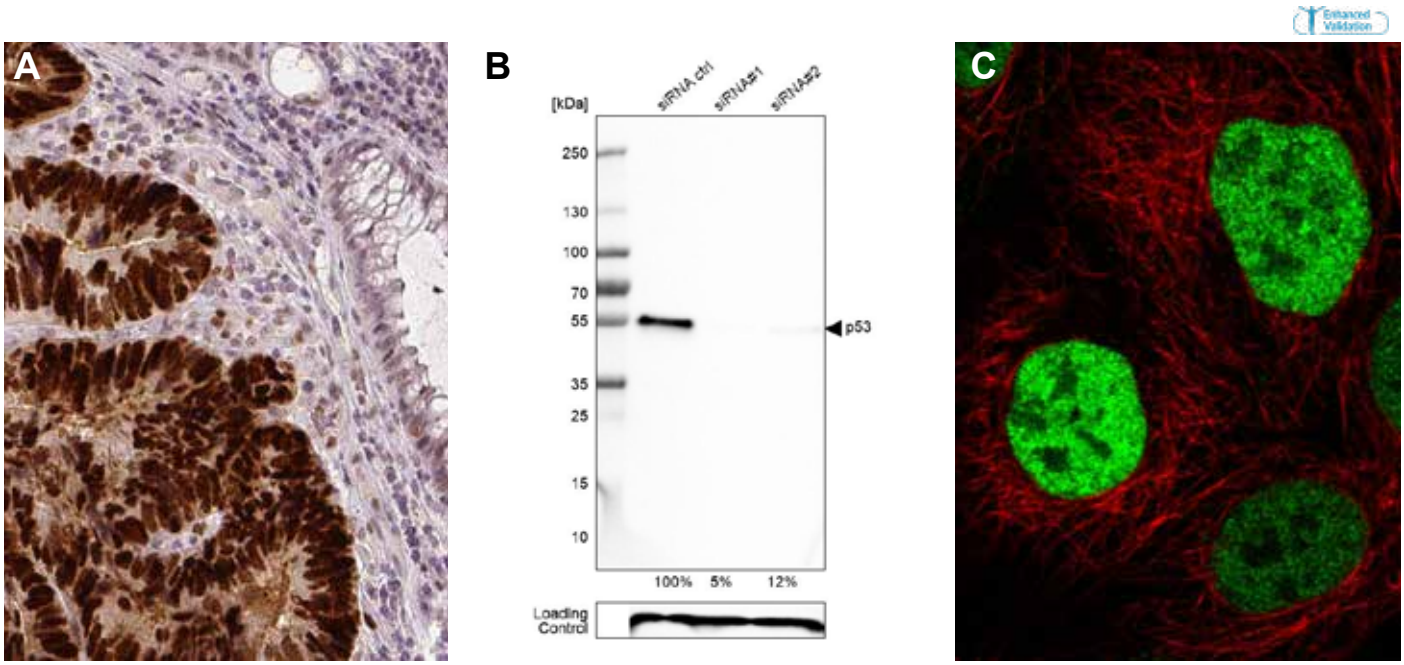

Figure 1.
A. IHC staining of human colorectal cancer using the Anti-p53 monoclonal antibody (AMAb90956) shows strong nuclear positivity in tumor cells but not in normal mucosa. **B.** WB analysis in U-251MG cells transfected with control siRNA, target-specific siRNA probe #1 and #2, using the Anti-p53 monoclonal antibody (AMAb90956). Remaining relative intensity is presented. Loading control: Anti-PPIB. **C.** ICC-IF staining in A431 cell line with the Anti-p53 monoclonal antibody (AMAb90956), showing cell cycle-dependent nuclear (without nucleoli) staining in green. Microtubules are visualized in red.

Cover image:
Multiplexed IHC-IF staining of human breast cancer section using the Anti-FOXA1 polyclonal antibody (HPA050505, nuclear staining in red) and the Anti-CDH1 monoclonal antibody (AMAb90865, membranous staining in green). DAPI is used as a counterstain (in blue).

Cell Cycle

Cancers lesions commonly originate from the aberrant cellular proliferation due to e.g., mutations or amplifications in the open-reading frames or regulatory domains of oncogenes and tumor suppressors that regulate cell growth. In addition, gross chromosomal alterations may generate fusion proteins with de novo transforming potential.

The cell cycle is a progressive set of molecular events that culminate into altered cell growth and mitotic rate, i.e., by which frequency one cell divides into two daughter cells. Cell cycle progression is positively regulated and enforced by a family of serine/threonine protein kinases collectively referred to as cyclin-dependent kinases (CDKs), which are activated by binding to respective cyclin partners and by phosphorylation.

These checkpoint pathways recognize and sense whether or not to initiate and/or promote cellular proliferation under a particular set of external and internal conditions, including timing.

Crucial components of these pathways are mainly proteins encoded by some of the checkpoint genes, including the tumor suppressor protein TP53.

Readings

Barnum K.J. and O'Connell M.J. (2014) Cell Cycle Regulation by Checkpoints. *Methods Mol Biol.* 1170: 29–40.

Feitelson M.A. et al. (2015) Sustained proliferation in cancer: mechanisms and novel therapeutic targets. *Semin Cancer Biol.* 35(Suppl): S25–S54

López-Sáez J.F. et al. (1998) Cell proliferation and cancer. *Histol Histopathol.*13(4):1197-214

Tobias O. and Sicinski P. (2017) Cell cycle proteins as promising targets in cancer therapy. *Nature Rev Cancer* 17:93-115

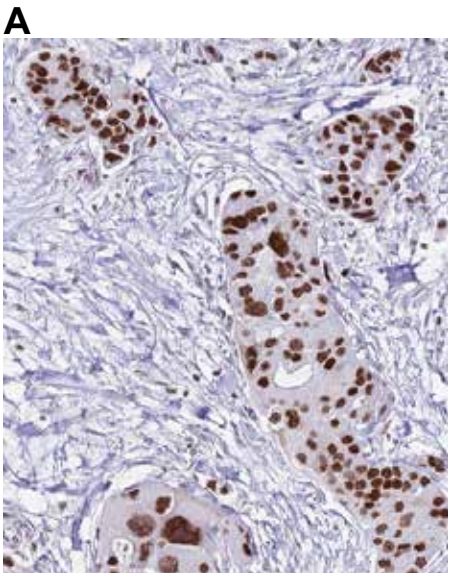

Figure 2.
A. IHC staining of human lung cancer with the Anti-PARP1 monoclonal antibody (AMAb90959) shows strong nuclear immunoreactivity, in brown. **B.** WB analysis in RT-4 cells transfected with control siRNA, target-specific siRNA probe #1 and #2, using the Anti-PARP1 monoclonal antibody (AMAb90959). Remaining relative intensity is presented. Loading control: Anti-GAPDH. **C.** ICC-IF staining in HeLa cell line with the Anti-PARP1 monoclonal antibody (AMAb90959), showing cell cycle-dependent nuclear staining in green. Microtubules are visualized in red.

Table 1. Suggested cell cycle markers from Atlas Antibodies

Product Name	Catalog No	Clonality	Application	Sequence Identity Mouse/Rat
Anti-AURKA	HPA002636	Polyclonal	IHC, WB, ICC-IF	66% / 67%
Anti-AURKB	HPA037708	Polyclonal	ICC-IF	55% / 53%
Anti-CCNA1/CyclinA1	HPA060646	Polyclonal	ICC-IF	54% / 53%
Anti-CCNB1/CyclinB1	HPA030741	Polyclonal	WB*, ICC-IF	80% / 78%
Anti-CCNB1/CyclinB1	HPA061448	Polyclonal	IHC*, WB, ICC-IF	72% / 68%
Anti-CCNB2/CyclinB2	HPA008873	Polyclonal	IHC*, WB, ICC-IF	81% / 27%
Anti-CCNB3/CyclinB3	HPA000496	Polyclonal	IHC*, ICC-IF	32% / 29%
Anti-CCND1/ CyclinD1	HPA027802	Polyclonal	ICC-IF	89% / 89%
Anti-CCND2/CyclinD2	HPA049138	Polyclonal	ICC-IF	74% / 70%
Anti-CCND2/CyclinD2	HPA054196	Polyclonal	ICC-IF	94% / 94%
Anti-CDK2	AMAb91497	Monoclonal	IHC*, WB, ICC-IF	100% / 100%
Anti-CDK4	AMAb91499	Monoclonal	IHC, WB, ICC-IF	94% / 94%
Anti-CDK5	HPA064535	Polyclonal	IHC*, ICC-IF	99% / 99%
Anti-CDK6	HPA002637	Polyclonal	IHC*, WB*, ICC-IF	92% / 92%
Anti-CDKN2A	HPA047838	Polyclonal	ICC-IF	44% / 43%
Anti-CDKN2B	HPA063327	Polyclonal	IHC	62% / 59%
Anti-CDKN2D	HPA043546	Polyclonal	ICC-IF	79% / 45%
Anti-CHEK1	HPA044364	Polyclonal	ICC-IF	93% / 92%
Anti-CHEK2	HPA001878	Polyclonal	IHC, WB*, ICC-IF	86% / 86%
Anti-CHEK2	AMAb91570	Monoclonal	IHC*, WB, ICC-IF	86% / 86%
Anti-E2F1	HPA008003	Polyclonal	IHC	76% / 76%
Anti-E2F1	HPA029735	Polyclonal	WB, ICC-IF	94% / 93%
Anti-p53	AMAb90956	Monoclonal	IHC*, WB*, ICC-IF	91% / 75%
Anti-p53	HPA051244	Polyclonal	WB*, ICC-IF	91% / 75%
Anti-PARP1	AMAb90959	Monoclonal	IHC, WB*, ICC-IF	95% / 94%
Anti-PARP1	AMAb90960	Monoclonal	IHC, WB*	95% / 94%
Anti-PARP1	HPA045168	Polyclonal	IHC, WB*, ICC-IF	95% / 94%
Anti-PLK1	HPA051638	Polyclonal	IHC	90% / 88%
Anti-PLK1	HPA053229	Polyclonal	IHC	97% / 85%
Anti-PLK1	AMAb91515	Monoclonal	WB	83% / 91%
Anti-RB1	HPA050082	Polyclonal	IHC	89% / 97%
Anti-RIF1	HPA036887	Polyclonal	IHC, ICC-IF	38% / 36%
Anti-RIF1	HPA036888	Polyclonal	IHC	52% / 49%

* Products with enhanced validation for indicated application

Cell Death

On the morphological basis, cell death can be achieved by apoptosis, necrosis, and autophagy. Apoptosis and autophagy are regarded as "programmed cell death" while necrosis is considered as "unprogrammed cell death" due to deregulated activity. Another type of cell death termed necroptosis, exhibits morphological features of both apoptosis and necrosis. An inappropriate level of cell death (either too little or too much) is a decisive factor in many human disease conditions, including cancer.

Apoptosis

Apoptosis (from the Greek meaning "falling off") remains the most well-studied mechanism of programmed cell death. Apoptosis is a regulated, ATP-dependent cell death mechanism crucial for the removal of surplus and/or aberrant cells. This process is ultimately dependent on the actions of the activated form of the protease caspase-3.

Apoptosis is important during development, organogenesis, and aging, and serves as a mechanism to maintain cellular homeostasis within tissues.

Cancer cells can surpass apoptosis by various mechanisms such as: upregulation of the anti-apoptotic (Bcl2) pathway or inhibition of the pro-apoptotic proteins through genetic or epigenetic mechanisms.

From a therapeutic point of view, apoptosis resistance is a major issue when it comes to treatment failure during cancer chemotherapy.

Autophagy

Autophagy (from the Greek "self-eating") plays a housekeeping role in removing misfolded/aggregated proteins and damaged organelles and depends upon their lysosomal proteolysis.

In healthy tissues, autophagy contributes to the homeostasis of the cells. The dysregulation of autophagy in cancer contributes to the protection of cells undergone malignant transformation. As a result, sustained survival and proliferation of tumor cells occur, supporting tumor growth, invasion, and metastasis.

Necroptosis

Necroptosis is the caspase-independent "cellular suicide" or "regulated" necrosis. It is an alternative mode of regulated cell death mimicking features of both apoptosis and necrosis. Pieces of evidence based on a mouse model reveal that the de-regulation of necroptosis is associated with pathological conditions like cancer. Necroptosis is primarily regulated by proteins RIPK1 and RIPK3, and it may trigger and amplify antitumor immunity in cancer therapy.

Readings

Glick D. et al. (2010) Autophagy: cellular and molecular mechanisms. *J Pathol.* 221(1): 3–12

Green DR. et al. (2002) A matter of life and death. *Cancer Cell.* 1(1):19-30

Guo JY., et al. (2013) Autophagy-mediated tumor promotion. *Cell* 155(6):1216-9

Kroemer G. et al. (2010) Autophagy and the integrated stress response. *Mol Cell.* 40(2):280-93

Lopez J. et al. (2015) Mitochondrial apoptosis: killing cancer using the enemy within. *Br J Cancer.* 112(6):957-62

McIlwain D.R. et al. (2013) Caspase Functions in Cell Death and Disease. *Cold Spring Harb Perspect Biol.* 5(4): a008656

Choi M.E. et al. (2019) Necroptosis: a crucial pathogenic mediator of human disease. *JCI Insight.* 4(15): e128834.

Table 2. Suggested cell death markers from Atlas Antibodies

Product Name	Catalog No	Clonality	Application	Sequence Identity Mouse/Rat
Anti-ANXA1	HPA011272	Polyclonal	IHC*, WB*, ICC-IF	92% / 89%
Anti-ATG5	HPA042973	Polyclonal	IHC, WB*, ICC-IF	96% / 94%
Anti-ATG5	AMAb91582	Monoclonal	IHC*, WB	96% / 94%
Anti-BAD	HPA028185	Polyclonal	IHC, WB, ICC-IF	59% / 57%
Anti-BAX	HPA027878	Polyclonal	IHC, WB*	90% / 88%
Anti-BAX	AMAb91490	Monoclonal	IHC, WB	79% / 79%
Anti-BCL2	AMAb91492	Monoclonal	IHC*, WB	100% / 94%
Anti-BCL2	HPA055295	Polyclonal	ICC-IF	60% / 55%
Anti-BID	HPA000722	Polyclonal	IHC*, WB*, ICC-IF	64% / 61%
Anti-CASP8	HPA005688	Polyclonal	IHC, WB	55% / 52%
Anti-CASP9	HPA046488	Polyclonal	IHC	79% / 78%
Anti-DIABLO	HPA001825	Polyclonal	IHC*, WB, ICC-IF	88% / 88%
Anti-FAS	HPA027444	Polyclonal	IHC*, WB, ICC-IF	52% / 48%
Anti-MAP1LC3A	HPA052474	Polyclonal	IHC	97% / 97%
Anti-mTOR	AMAb91508	Monoclonal	WB	100% / 96%
Anti-p53	AMAb90956	Monoclonal	IHC*, WB*, ICC-IF	91% / 75%
Anti-p53	HPA051244	Polyclonal	WB*, ICC-IF	91% / 75%
Anti-PARP1	AMAb90959	Monoclonal	IHC, WB*, ICC-IF	95% / 94%
Anti-PARP1	AMAb90960	Monoclonal	IHC, WB*	95% / 94%
Anti-PARP1	HPA045168	Polyclonal	IHC, WB*, ICC-IF	95% / 94%
Anti-PDCD1	AMAb91197	Monoclonal	IHC, WB	66% / 63%
Anti-PIK3CA	AMAb91513	Monoclonal	WB	96% / 96%
Anti-PIK3CA	AMAb91514	Monoclonal	IHC*	96% / 96%
Anti-PIK3CB	AMAb91585	Monoclonal	IHC, WB, ICC-IF	84% / 84%
Anti-RIPK1	HPA015257	Polyclonal	IHC*, WB, ICC-IF	67% / 63%
Anti-RIPK2	HPA015273	Polyclonal	IHC, WB, ICC-IF	73% / 73%
Anti-RIPK3	HPA055087	Polyclonal	IHC, WB	38% / 38%
Anti-ULK1	HPA063990	Polyclonal	ICC-IF	87% / 87%

* Products with enhanced validation for indicated application

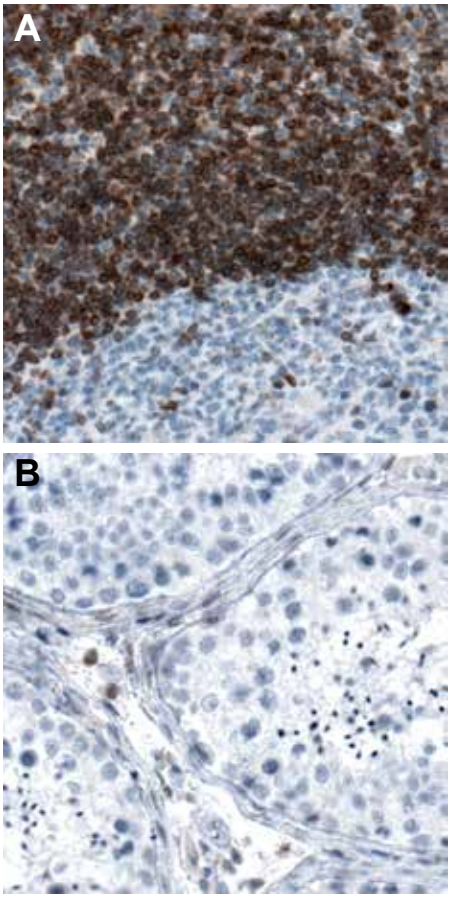

Figure 3.
A. IHC staining of the human tonsil with the Anti-BCL2 monoclonal antibody (AMAb91492) shows strong positivity in non-germinal center cells.
B. IHC staining of the human testis with the Anti-BCL2 monoclonal antibody (AMAb91492) shows no positivity in cells in seminiferous tubules, as expected (negative control).

Metastasis

A unique and clinically decisive feature of cancer cells is their capability to disseminate from their point of origin invading secondary and distal tissue sites, a set of events known as metastasis.

The metastasis cascade refers to the sequential occurrences enabling cancer cells to detach from each other in situ and escape from the primary tumor site. The final destination of metastatic cells is non-random and depends on both tissue vascularization and the expression of particular homing proteins in the respective cell surfaces.

The metastatic tumor cells lose their adhesive properties and contacts with the basement membrane. This process depends on e.g., expression of matrix metalloproteases (MMPs) which degrade the basement membrane, allowing cells to disseminate, reach their distal destinations via vascular routes (blood and lymph vessels) and, finally, to converge the formation of clinically detectable micro- or macroscopic secondary tumors in distal organs (metastases).

Upon exit from the primary site and during dissemination, metastatic cells are reprogrammed into a mesenchymal phenotype through the so-called Epithelial-to-Mesenchymal-Transition (EMT). Upon EMT, cancers of epithelial origin acquire molecular signatures usually associated with a “stem cell-like” phenotype, which includes, for instance, induction of TGF-beta, transcription factors Snail1- and -2, Twist, as well as the intermediate filament protein vimentin.

The histopathological analysis may enable the distinction of the invading tumor cells from the healthy local tissue. Sometimes, the presence of invading metastases is revealed by the mere differences in size and nuclear morphology (degree of pleomorphism) of the tumor cells versus the healthy surrounding tissue. To that end, conventional histochemical staining like H&E is employed.

However, to define the origin of the metastatic tumor cells using only conventional histopathology can be challenging. Therefore, specific phenotypic IHC-markers like e.g., cytokeratins KRT7 and KRT20 can be used, often as a panel of different markers.

Table 3. Suggested metastasis markers from Atlas Antibodies

Product Name	Catalog No	Clonality	Application	Sequence Identity Mouse/Rat
Anti-CEACAM5	HPA019758	Polyclonal	IHC*, WB, ICC-IF	50% / 49%
Anti-KRT20	HPA027236	Polyclonal	IHC*, WB*	83% / 76%
Anti-KRT7	HPA007272	Polyclonal	IHC*, WB*	90% / 90%
Anti-MMP9	AMAb90804	Monoclonal	IHC, WB	80% / 78%
Anti-MMP9	AMAb90805	Monoclonal	IHC, WB	80% / 78%
Anti-MMP9	AMAb90806	Monoclonal	IHC	80% / 78%
Anti-MMP9	HPA001238	Polyclonal	IHC*, ICC-IF	80% / 78%
Anti-MMP9	HPA063909	Polyclonal	IHC*, WB*	58% / 57%
Anti-SNAI1	AMAb91215	Monoclonal	IHC*, ICC-IF	82% / 82%
Anti-SNAI1	HPA069985	Polyclonal	IHC	82% / 82%
Anti-TTF1	HPA054837	Polyclonal	IHC, WB, ICC-IF	44% / 44%
Anti-Twist2	HPA062870	Polyclonal	ICC-IF	100% / 100%
Anti-VIM	HPA001762	Polyclonal	IHC*, WB*, ICC-IF	99% / 99%
Anti-VIM	AMAb90516	Monoclonal	IHC, WB*	99% / 99%

* Products with enhanced validation for indicated application

Readings

Wittekind C. and Neid M. (2005) Cancer Invasion and Metastasis. *Oncology* 69:14–16

Seyfried T.N. and Huysentruyt L.C. (2013) On the Origin of Cancer Metastasis. *Crit Rev Oncolog*.18(1-2): 43–73

Wang R.A. et al. (2015) Reasons for cancer metastasis: A holistic perspective. *Mol Clin Oncol*. 3(6): 1199–1202

Lee S.L.C. et al. (2009) Hypoxia-induced pathological angiogenesis mediates tumor cell dissemination, invasion, and metastasis in a zebrafish tumor model. *PNAS* 106(46):19485-19490

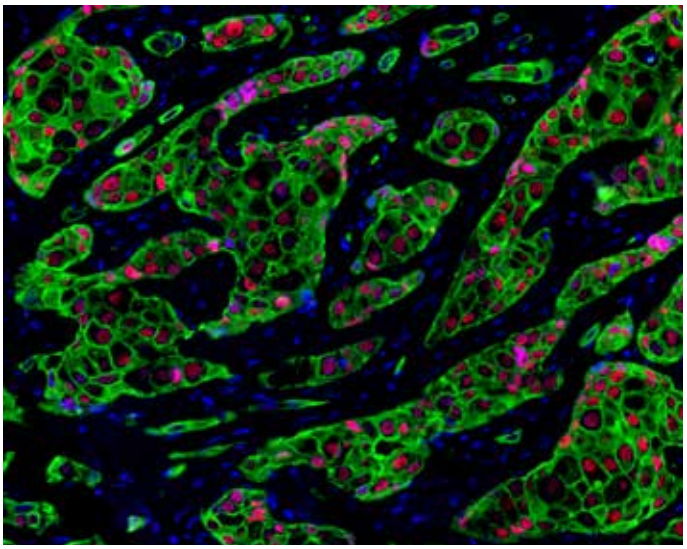

Figure 4. Multiplexed IHC-IF staining of a human breast cancer section using the Anti-AR monoclonal antibody (AMAb91547, nuclear staining in red) and the Anti-KRT7 polyclonal antibody (HPA007272, cytoplasmatic staining in green). DAPI is used as a counterstain (in blue).

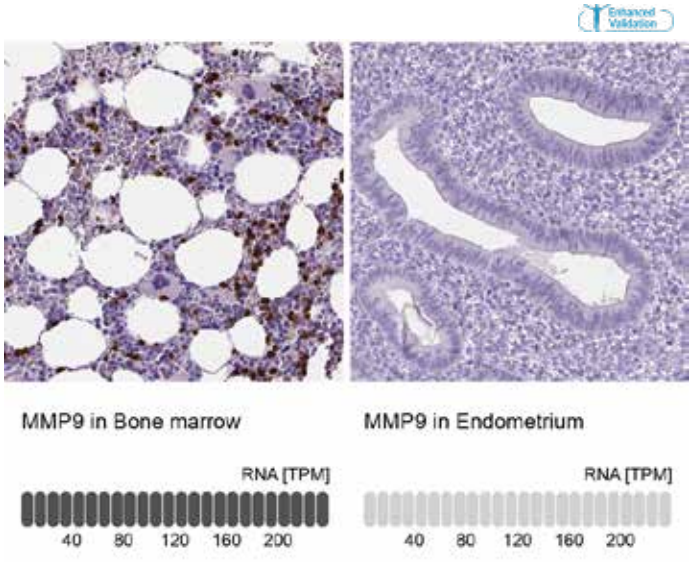

Figure 5. IHC analysis in the human bone marrow and endometrium tissues using the Anti-MMP9 monoclonal antibody (AMAb90804). Corresponding MMP9 RNA-seq data are presented for the same tissues.

Tumor Angiogenesis and Lymphoangiogenesis

Cancer cells differ from healthy cells and benign tumor cells in their metabolic characteristics. Paradoxically, despite sufficient oxygen (O_2) levels, aggressive cancers preferentially produce lactate.

This distinguishing feature is known as the "Warburg effect." The metabolic state of these tumors arises from changes in the tumor microenvironment (TME), coupled among other things, to O_2 availability. Ultimately, the increased growth and metabolic demand of malignant tumors lead to intra-tumoral hypoxia (lowered partial O_2 pressure).

Hypoxia is associated with metabolic reprogramming and enhanced glycolysis. A chief mechanism regulating aerobic glycolysis in cancer cells involves the Hypoxia-Inducing Factor (HIF-1alpha), which is responsible for the altered state of metabolism in cancer by triggering the transcription of a multitude of factors.

Angiogenesis is the life-long formation of blood vessels from the existing vasculature. It occurs throughout life in both health and disease. Neo-angiogenesis, including lymphoangiogenesis (formation of lymph vessels), plays a significant role in both tumor growth and metastasis.

In fact, like all living cells, cancer cells strongly depend on an adequate supply of O_2 and nutrients and the removal of waste products for their survival. Endogenous regulators of angiogenesis include, among others, growth factors, cytokines, proteases, protease inhibitors, and oncogenes.

Recognition that control of angiogenesis (decreasing or inhibiting) could have therapeutic value has stimulated considerable interest during the past 40 years.

However, the clinical outcome of this therapeutic approach has been limited, not least due to resistance development. Also, anti-angiogenic therapy may be of a disadvantage because many drugs reach the tumor through a vascular route.

Readings

Lu J. et al. (2015) The Warburg effect in tumor progression: mitochondrial oxidative metabolism as an anti-metastasis mechanism. *Cancer Lett.* 356 (2A):156-64

Rajabi M. and Mousa S.A. (2017) The Role of Angiogenesis in Cancer Treatment. *Biomedicines* 5(2):34

Romero-Garcia S. et al. (2011) Tumor cell metabolism - an integral view. *Cancer Biol Ther.*12(11):939–948

Tonini T. et al. (2003) Review: Molecular basis of angiogenesis and cancer. *Oncogene* 22:6549–6556

Yin Z. et al. (2019) Targeting T cell metabolism in the tumor microenvironment: an anti-cancer therapeutic strategy. *J Exp&Clin Cancer Res*. 38:403

Table 4. Suggested tumor- and lympho-angiogenesis markers from Atlas Antibodies

Product Name	Catalog No	Clonality	Application	Sequence Identity Mouse/Rat
Anti-Akt1	AMAb90834	Monoclonal	WB, ICC-IF	97% / 97%
Anti-Akt1	AMAb90835	Monoclonal	WB	97% / 97%
Anti-CD34	HPA036722	Polyclonal	IHC	63% / 56%
Anti-EGFR	AMAb90816	Monoclonal	IHC, WB	91% / 90%
Anti-ENG	AMAb90925	Monoclonal	IHC	66% / 22%
Anti-EPAS1	HPA031200	Polyclonal	ICC-IF	95% / 93%
Anti-FLT1	AMAb90704	Monoclonal	IHC*, WB	80% / 82%
Anti-FLT4	HPA067906	Polyclonal	IHC	75% / 74%
Anti-GAPDH	AMAb91152	Monoclonal	IHC, WB*	94% / 92%
Anti-GAPDH	AMAb91153	Monoclonal	IHC, WB*, ICC-IF	94% / 92%
Anti-GAPDH	HPA040067	Polyclonal	IHC, WB*, ICC-IF	94% / 92%
Anti-GAPDH	HPA061280	Polyclonal	WB*, ICC-IF	92% / 90%
Anti-HIF1A	HPA000907	Polyclonal	ICC-IF	88% / 87%
Anti-HIF1A	HPA001275	Polyclonal	IHC	88% / 87%
Anti-IDH1	AMAb90578	Monoclonal	IHC, WB*, ICC-IF	95% / 95%
Anti-IDH1	HPA035248	Polyclonal	IHC*, WB*	95% / 95%
Anti-IDH1	HPA057936	Polyclonal	IHC*, WB*	95% / 92%
Anti-IDH2	HPA007831	Polyclonal	IHC*, WB*, ICC-IF	95% / 95%
Anti-LYVE1	HPA042953	Polyclonal	IHC, WB	63% / 61%
Anti-NES	AMAb90556	Monoclonal	IHC, WB*, ICC-IF	47% / 42%
Anti-NES	HPA007007	Polyclonal	IHC*, WB*	47% / 42%
Anti-PDGFRB	HPA028499	Polyclonal	WB, ICC-IF	76% / 76%
Anti-VEGFA	HPA069116	Polyclonal	IHC	86% / 88%
Anti-VEGFD	HPA027342	Polyclonal	IHC, WB*	86% / 89%
Anti-VHL	HPA031631	Polyclonal	ICC-IF	49% / 43%
Anti-vWF	AMAb90928	Monoclonal	IHC, WB	80% / 80%
Anti-vWF	HPA001815	Polyclonal	IHC	80% / 80%
Anti-vWF	HPA002082	Polyclonal	IHC	82% / 78%
Anti-vWF	AMAb90931	Monoclonal	IHC, WB	80% / 80%

* Products with enhanced validation for indicated application

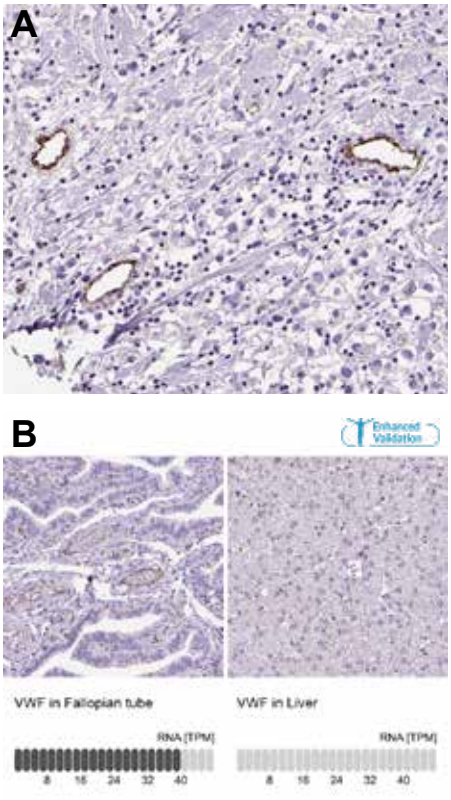

Figure 6.
A. IHC analysis in human breast cancer using the Anti-VWF monoclonal antibody (AMAb90931).
B. IHC analysis in human fallopian tube and liver using the Anti-VWF monoclonal antibody (AMAb90931). Corresponding VWF RNA-seq data are presented for the same tissues.

Tumor Proliferation

Abnormal proliferation is one of the cardinal features of cancer and is indispensable for tumor development and progression. The processes that restrain healthy regulated cell growth are often compromised or lost in cancer.

Many aggressive tumors, particularly carcinomas, have a very rapid proliferation rate while others, like some neuroendocrine tumors, grow very slowly. Hyperproliferation is often triggered by constitutively activated signal transduction pathways that promote uncontrolled proliferation.

These include growth factor signaling pathways mediated by, for example, tyrosine kinase receptors (EGFR, PDGFR, IGF-1R, Wnt, Bcr-Abl, PI3K/Akt, etc.).

The fact that cancer cells show increased proliferation rate, makes them particularly sensitive to cytostatic agents. One of the most common therapy in cancer is the use of cytotoxic (cytostatic) drugs.

Other therapeutic options may include small molecules, e.g., tyrosine kinase inhibitors or therapeutic antibodies like trastuzumab (Herceptin) against HER2.

Table 5. Suggested tumor proliferation markers from Atlas Antibodies

Product Name	Catalog No	Clonality	Application	Sequence Identity Mouse/Rat
Anti-ABL1	HPA028409	Polyclonal	IHC, ICC-IF	75% / 77%
Anti-BRAF	AMAb91257	Monoclonal	IHC, WB	93% / 92%
Anti-BRAF	AMAb91258	Monoclonal	IHC, WB	93% / 92%
Anti-BRAF	HPA001328	Polyclonal	IHC, WB	93% / 92%
Anti-BRAF	HPA071048	Polyclonal	WB, ICC-IF	100% / 100%
Anti-CSF1R	HPA012323	Polyclonal	IHC, WB, ICC-IF	58% / 61%
Anti-EGFR	AMAb90816	Monoclonal	IHC, WB	90% / 91%
Anti-EGFR	HPA018530	Polyclonal	IHC*, WB, ICC-IF	84% / 82%
Anti-ERBB3/HER3	HPA045396	Polyclonal	IHC, WB	78% / 77%
Anti-ESR1	AMAb90867	Monoclonal	IHC, WB*, ICC-IF	88% / 87%
Anti-FLT1	AMAb90703	Monoclonal	IHC*	80% / 82%
Anti-FLT1	AMAb90704	Monoclonal	IHC*, WB	80% / 82%
Anti-FLT4	HPA067906	Polyclonal	IHC	75% / 74%
Anti-FOS	HPA018531	Polyclonal	IHC, ICC-IF	94% / 94%
Anti-HER2	AMAb90627	Monoclonal	IHC, WB	84% / 85%
Anti-HER2	AMAb90628	Monoclonal	IHC, WB, ICC-IF	84% / 85%
Anti-HER2	HPA001383	Polyclonal	IHC, WB, ICC-IF	82% / 81%
Anti-JUN	HPA059474	Polyclonal	ICC-IF	97% / 95%
Anti-KIT	HPA004471	Polyclonal	IHC*	66% / 72%
Anti-KIT	AMAb90901	Monoclonal	IHC, WB	66% / 72%
Anti-MKI67	AMAb90870	Monoclonal	IHC, ICC-IF	68% / 68%
Anti-MYC	HPA055893	Polyclonal	IHC, ICC-IF	92% / 89%
Anti-MYCN	HPA057420	Polyclonal	ICC-IF	87% / 87%
Anti-NFKB2	HPA008422	Polyclonal	IHC*, WB*, ICC-IF	93% / 92%
Anti-NFKB2	HPA023900	Polyclonal	IHC*, WB*	96% / 96%
Anti-PCNA	HPA030522	Polyclonal	IHC*, WB*, ICC-IF	99% / 100%
Anti-PDGFRB	HPA028499	Polyclonal	WB, ICC-IF	76% / 76%
Anti-PGR	AMAb91529	Monoclonal	IHC*, ICC-IF	67% / 68%
Anti-PTEN	HPA031335	Polyclonal	WB, ICC-IF	100% / 100%
Anti-SMAD2	AMAb91520	Monoclonal	IHC*, WB, ICC-IF	94% / 94%
Anti-SMAD3	HPA046386	Polyclonal	ICC-IF	100% / 100%
Anti-SMAD3	HPA067203	Polyclonal	IHC, WB	100% / 100%
Anti-SMAD4	HPA019154	Polyclonal	IHC, WB, ICC-IF	94% / 95%
Anti-SMAD4	AMAb91594	Monoclonal	IHC, WB	94% / 95%
Anti-SMAD7	HPA028897	Polyclonal	IHC, ICC-IF	99% / 99%
Anti-TGFA	HPA042297	Polyclonal	IHC	93% / 93%
Anti-TGFB1	HPA017019	Polyclonal	IHC*, WB	88% / 87%
Anti-VEGFA	HPA069116	Polyclonal	IHC	86% / 88%

* Products with enhanced validation for indicated application

Readings

Feitelson MA. et al. (2015) Sustained proliferation in cancer: Mechanisms and novel therapeutic targets. *Seminars in Cancer Biology* 35, S25-S54

Madewell BR. et al. (2001) Cellular Proliferation in Tumors: A Review of Methods,Interpretation, and Clinical Applications. *J Vet Intern Med*15:334–340

Whitfield ML. et al. (2006) Common markers of proliferation. *Nat Rev Cancer* 6, 99–106

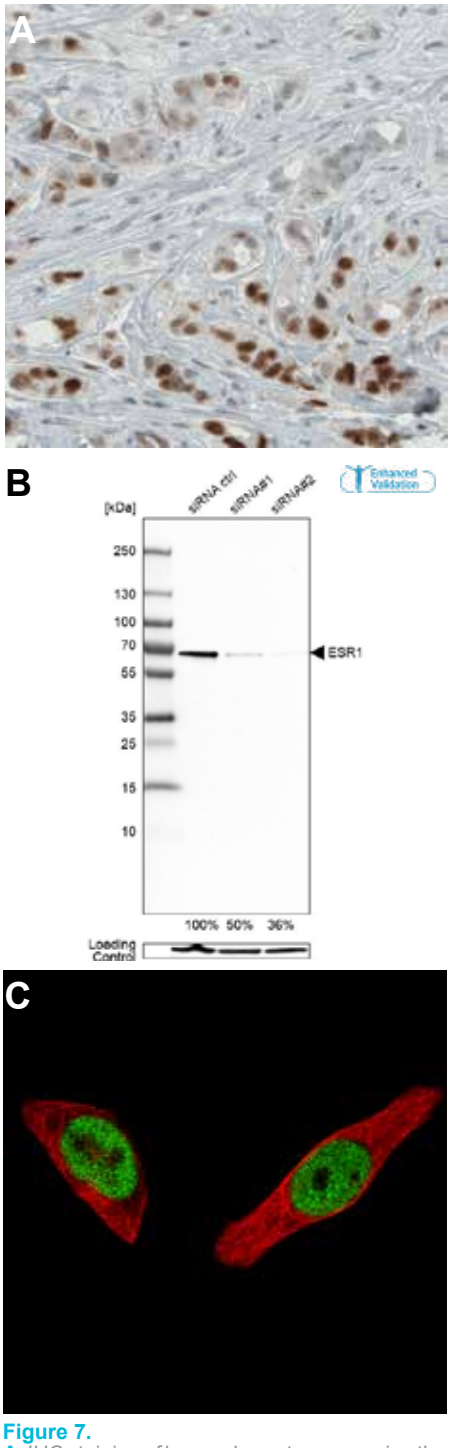

Figure 7.
A. IHC staining of human breast cancer using the Anti-ESR1 monoclonal antibody (AMAb90867) shows strong nuclear immunoreactivity in a subset of tumor cells.
B. WB analysis in MCF-7 cells transfected with control siRNA, target-specific siRNA probe #1 and #2, using the Anti-ESR1 antibody (AMAb90867). Remaining relative intensity is presented. Loading control: Anti-GAPDH.
C. ICC-IF staining of MCF-7 cells using the Anti-ESR1 antibody (AMAb90867), showing specific staining in the nucleoplasm in green. Microtubules are visualized in red.

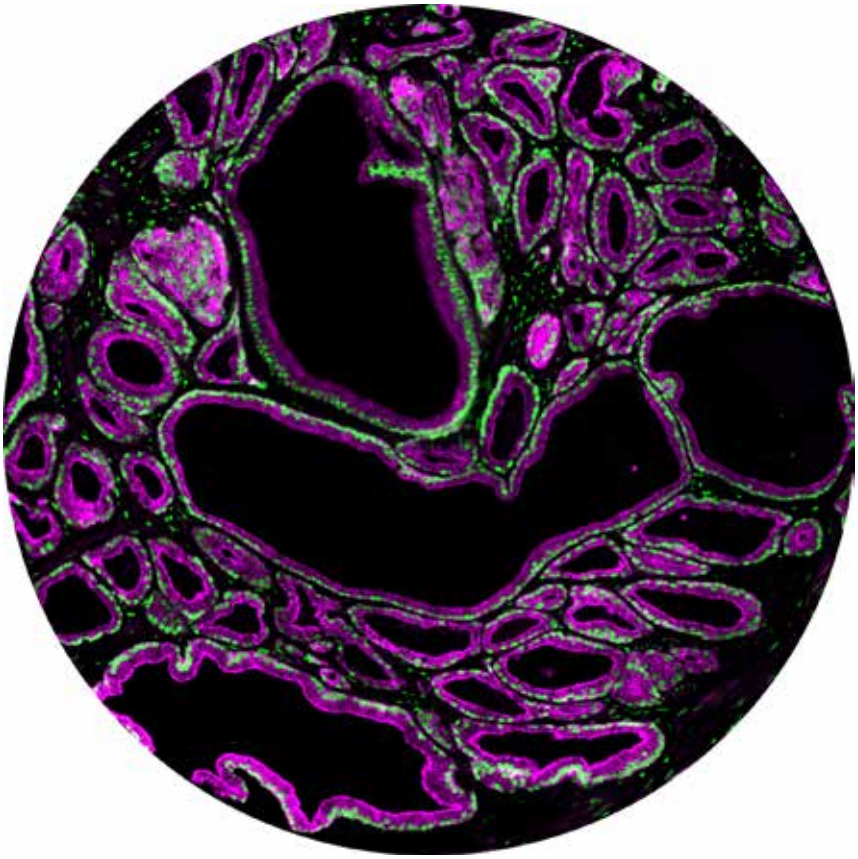

image: Multiplexed IHC-IF staining of a human prostate cancer section using the Anti-AR monoclonal antibody AMAb91547 (nucleus, in green) and the Anti-KRT7 polyclonal antibody HPA007272 (cytoplasm, in magenta).

Enhanced Validation

At Atlas Antibodies, we take great care to validate our antibodies in IHC, WB, and ICC-IF. Enhanced Validation is performed as an additional layer of security in an application and context-specific manner. Enhanced validation offers increased security of antibody specificity in a defined context. This is ensured by using the most relevant validation method for each combination of protein, sample, and application. Enhanced Validation follows the guidelines proposed by the International Working Group for Antibody Validation (IWGAV).

PrecisA MONOCLONALS
DESIGNED BY RESEARCHERS FOR RESEARCHERS

PrecisA Monoclonals™ are mouse monoclonal primary antibodies developed against a number of carefully selected targets. Clones are selected to recognize only unique non-overlapping epitopes and isotypes.

AAA POLYCLONALS
Produced by researchers. For researchers.

Triple A Polyclonals™ are rabbit polyclonal primary antibodies developed within the Human Protein Atlas project. IHC characterization data from 44 normal tissues and 20 cancers is available on the Human Protein Atlas portal.

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