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AIMM Applied Immunohistochemistry & Molecular Morphology
OFFICIAL PUBLICATION OF THE INTERNATIONAL SOCIETY FOR IMMUNOHISTOCHEMISTRY & MOLECULAR MORPHOLOGY

Next-Generation Immunohistochemistry: How to Build Highly Effective Diagnostic Tests in Anatomic Pathology

Andrew M Bellizzi, M.D.
Department of Pathology
University of Iowa Hospitals and Clinics
andrew-bellizzi@uiowa.edu
@IHC_guy

ISIMm
International Society for IHC and Molecular Morphology

Biography

- Director, UIHC Immunohistochemistry Lab
- Chair, CAP Immunohistochemistry Committee
- Assoc. Ed., *Appl Immunohistochem Mol Morphol*
- Expert Panel Member, CAP Center Guideline: *Principles of Analytic Validation of IHC Assays Update*
- Secretary-Treasurer, International Society for Immunohistochemistry and Molecular Morphology
- Director, GI Pathology at the University of Iowa
- Director, GI Pathology Fellowship

Value Proposition

- Everybody benefits from smart, technically sound immunohistochemistry
- I'll be your immunohistochemistry concierge
  - andrew-bellizzi@uiowa.edu
  - @IHC_guy
- Trainees
- IHC Lab Director
- Clinical Faculty
- Scientists

Asian Journal of Urology (2019) 6, 10–25

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journal homepage: www.elsevier.com/locate/ajur

Review

If this is true, what does it imply? How end-user antibody validation facilitates insights into biology and disease

Karen S. Sfanos [a], Srinivasan Yegnasubramanian [b], William G. Nelson [b], Tamara L. Lotan [a], Ibrahim Kulac [c], Jessica L. Hicks [a], Qizhi Zheng [a], Charles J. Bieberich [d], Michael C. Haffner [a], Angelo M. De Marzo [a,*]

[a] Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD, USA
[b] Department of Oncology, The Johns Hopkins University School of Medicine, Baltimore, MD, USA
[c] Department of Pathology, Koc Universitesi Tip Fakultesi, Istanbul, Turkey
[d] Department of Biological Sciences, University of Maryland Baltimore County, Baltimore, MD, USA

Received 31 July 2018; received in revised form 8 November 2018; accepted 12 November 2018
Available online 12 December 2018

KEYWORDS
Prostate cancer;
Antibodies;
Immunohistochemistry

Abstract Antibodies are employed ubiquitously in biomedical sciences, including for diagnostics and therapeutics. One of the most important uses is for immunohistochemical (IHC) staining, a process that has been improving and evolving over decades. IHC is useful when properly employed, yet misuse of the method is widespread and contributes to the "reproducibility crisis" in science. We report some of the common problems encountered with IHC assays, and direct readers to a wealth of literature documenting and providing some solutions to this problem. We also describe a series of vignettes that include our approach to analytical validation of antibodies and IHC assays that have facilitated a number of biological insights into prostate cancer and the refutation of a controversial association of a viral etiology in gliomas. We postulate that a great deal of the problem with lack of accuracy in IHC assays stems from the lack of awareness by researchers for the critical necessity for end-users to validate IHC antibodies and assays in their laboratories, regardless of manufacturer claims or past publications. We suggest that one reason for the pervasive lack of end-user validation for research antibodies is that researchers fail to realize that there are two general classes of antibodies employed in IHC. First, there are antibodies that are "clinical grade" reagents used by pathologists to help render diagnoses that influence patient treatment. Such diagnostic antibodies, which tend to be highly validated prior to clinical implementation, are in the vast minority (*e.g.* < 500). The other main class of antibodies are "research grade" antibodies (now

* Corresponding author.
E-mail address: ademarz@jhmi.edu (A.M. De Marzo).
Peer review under responsibility of Second Military Medical University.

https://doi.org/10.1016/j.ajur.2018.11.006

A proposal for validation of antibodies

Mathias Uhlen[1], Anita Bandrowski[2], Steven Carr[3], Aled Edwards[4], Jan Ellenberg[5], Emma Lundberg[1], David L Rimm[6], Henry Rodriguez[7], Tara Hiltke[7], Michael Snyder[8] & Tadashi Yamamoto[9]

We convened an *ad hoc* International Working Group for Antibody Validation in order to formulate the best approaches for validating antibodies used in common research applications and to provide guidelines that ensure antibody reproducibility. We recommend five conceptual 'pillars' for antibody validation to be used in an application-specific manner.

Antibodies are among the most frequently used tools in basic science research and in clinical assays. Despite their widespread use, as well as extensive and valuable discourse in the literature[1–6], a comprehensive scientific framework for antibody validation across research applications is lacking. As a result, the quality and consistency of data generated through the use of antibodies vary greatly. This poses an impediment to the rigor and reproducibility that are the cornerstones of the advancement of science.

The extensive discussion of antibody validation in the literature indicates a collective need for standards to validate antibody specificity and reproducibility, as well as a need for adequate reporting practices. For example, in 2010, Bourbeillon and colleagues[4] introduced the minimum information about a protein affinity reagent (MIAPAR) proposal. This proposal was meant to formalize a standard for how to report information about affinity binder reagents so that the correct reagent for a particular target could be selected for a specific application. The MIAPAR proposal is a useful guide for this purpose; however, it does not include explicit recommendations for the experimental approaches best suited to support validation of antibody specificity in particular applications.

Immunoreagents are used in a range of applications. According to the antibody reagent portal Antibodypedia (http://www.antibodypedia.com; **Supplementary Fig. 1**), their most common application is in western blot assays (immunoblotting), followed by immunohistochemistry and immunocytochemistry. In addition, the sandwich assay (e.g., ELISA), although it encompasses only a low percentage of overall antibody use, is an important application from a clinical perspective.

It is essential to note that samples are treated substantially differently in preparation for different antibody-based assays (**Supplementary Table 1**). Proteins are typically in near-native form for flow a simple and single benchmark for characterizing antibody performance for the full range of possible applications. Indeed, extensive characterization of antibody performance in western blotting may indicate nothing about the performance of the same antibody in an ELISA assay, where the antibody must recognize the epitope within the protein's native conformation. Likewise, an antibody may specifically recognize a cell surface protein in unfixed hematopoietic cells in flow cytometry but fail to bind the same protein in fixed liver tissue processed for immunohistochemistry. Therefore, approaches for antibody validation must be carried out in an application- and context-specific manner.

The International Working Group for Antibody Validation (IWGAV) was convened as an *ad hoc* committee of international scientists with diverse research interests but the shared goal of improving standards for antibody use and validation. Here, we propose a set of standard guidelines for validating antibodies, guidelines

Table 1 | Proposed conceptual pillars for validation of antibodies

Validation strategy	Genetic	Orthogonal	Independent antibody	Tagged protein expression	IMS
Validation principle	The expression of the target protein is eliminated or significantly reduced by genome editing or RNA interference	Expression of the target protein is compared with an antibody-independent method	Expression of the target protein is compared using two antibodies with nonoverlapping epitopes	The target protein is expressed using a tag, preferably expressed at endogenous levels	The target protein is captured using an antibody and analyzed using MS
Validation criteria	Elimination or significant reduction in antibody labeling after gene disruption or mRNA knockdown	Significant correlation of protein levels detected by an antibody and an orthogonal method (e.g., MS)	Significant correlation of protein levels detected by two different antibodies recognizing independent regions of the same target protein	Significant correlation between antibody labeling and detection of the epitope tag	Target protein peptides among the most abundant detected by MS following immunocapture
Suitable for these applications	WB, IHC, ICC, FS, SA, IP/ChIP, RP	WB, IHC, ICC, FS, SA, RP	WB, IHC, ICC, FS, SA, IP/ChIP, RP	WB, IHC, ICC, FS	IP/ChIP

WB, western blot; IHC, immunohistochemistry; ICC, immunocytochemistry, including immunofluorescence microscopy; FS, flow sorting and analysis of cells; SA, sandwich assays, including ELISA; IP, immunoprecipitation; ChIP, chromatin immunoprecipitation; and RP, reverse-phase protein arrays.

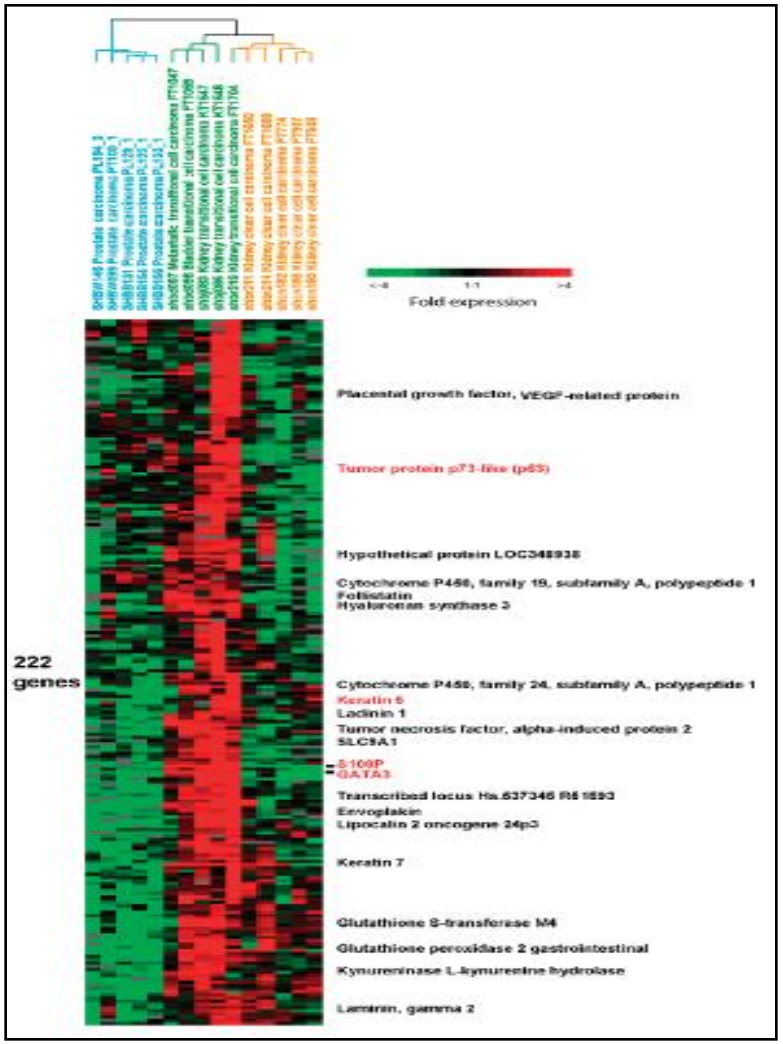
Outline

- Next-Generation Immunohistochemistry
- Selection, Optimization, Validation
- Specific Examples
  - OTC
  - IGF2
  - PAX1
  - mGluR1
  - HEY1 (N-terminus)
  - GATA-4

Next-Generation Immunohistochemistry

- Mine molecular genetic and developmental biology literature to find:
  - Markers identified by **gene expression profiling**
  - Protein correlates of **molecular genetic events**
  - Lineage-restricted **transcription factors**
- Bottom line: our markers keep getting better

Gene Expression Profiling Comparing Urothelial, Kidney, and Prostate Cancer



Higgins JPT, et al. *Am J Surg Pathol*. 2007 May;31(5):673-80.

LETTERS

nature genetics

Identification of recurrent *NAB2-STAT6* gene fusions in solitary fibrous tumor by integrative sequencing

Dan R Robinson[1,2,12], Yi-Mi Wu[1,2,12], Shanker Kalyana-Sundaram[1–3], Xuhong Cao[1,4], Robert J Lonigro[1,5], Yun-Shao Sung[6], Chun-Liang Chen[6], Lei Zhang[6], Rui Wang[1,2], Fengyun Su[1,2], Matthew K Iyer[1,7], Sameek Roychowdhury[1,8], Javed Siddiqui[1,2], Kenneth J Pienta[1,5,8,9], Lakshmi P Kunju[1,2], Moshe Talpaz[5,8], Juan Miguel Mosquera[10], Samuel Singer[11], Scott M Schuetze[5,8], Cristina R Antonescu[6] & Arul M Chinnaiyan[1,2,4,5,7,9]

Robinson DR, et al. *Nat Genet*. 2013 Feb;45(2):180-5.

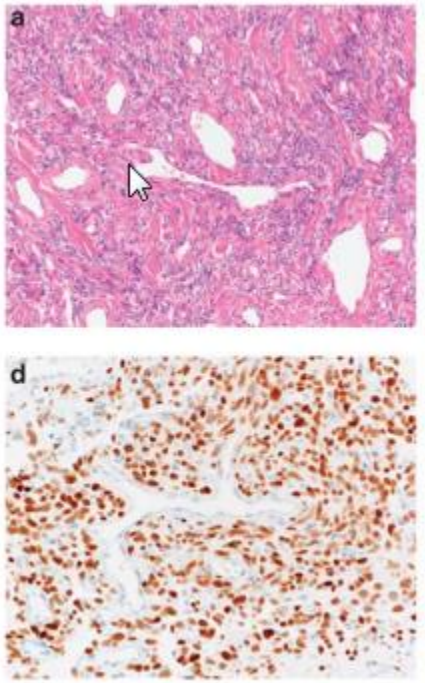
MODERN PATHOLOGY (2014) 27, 390–395

Nuclear expression of STAT6 distinguishes solitary fibrous tumor from histologic mimics

Leona A Doyle[1], Marina Vivero[1], Christopher DM Fletcher[1], Fredrik Mertens[2] and Jason L Hornick[1]

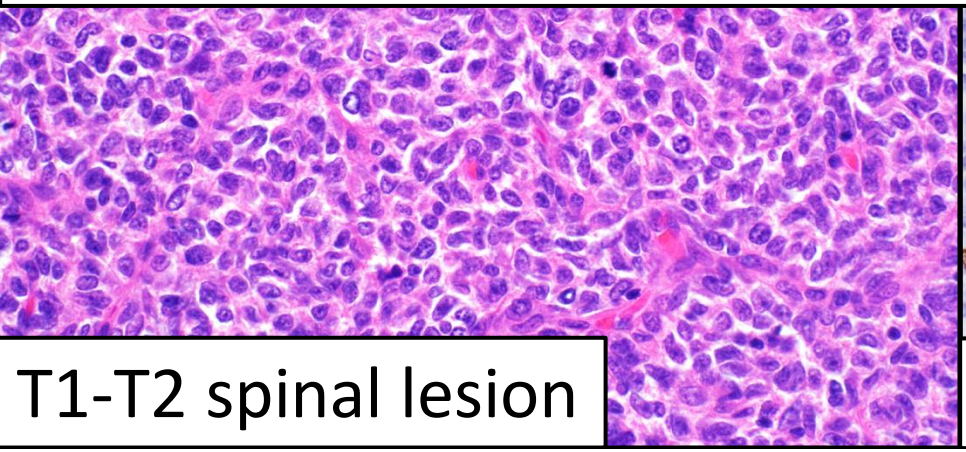
[1]Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA and [2]Department of Clinical Genetics, University and Regional Laboratories, Skåne University Hospital, Lund University, Lund, Sweden

Immunohistochemistry for STAT6 was performed on 4-μm-thick formalin-fixed paraffin-embedded tissue sections following pressure cooker antigen retrieval (0.01 M citrate buffer; pH, 6.0) using a rabbit polyclonal antibody directed against the C terminus of STAT6 (1:1000; sc-621; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Appropriate

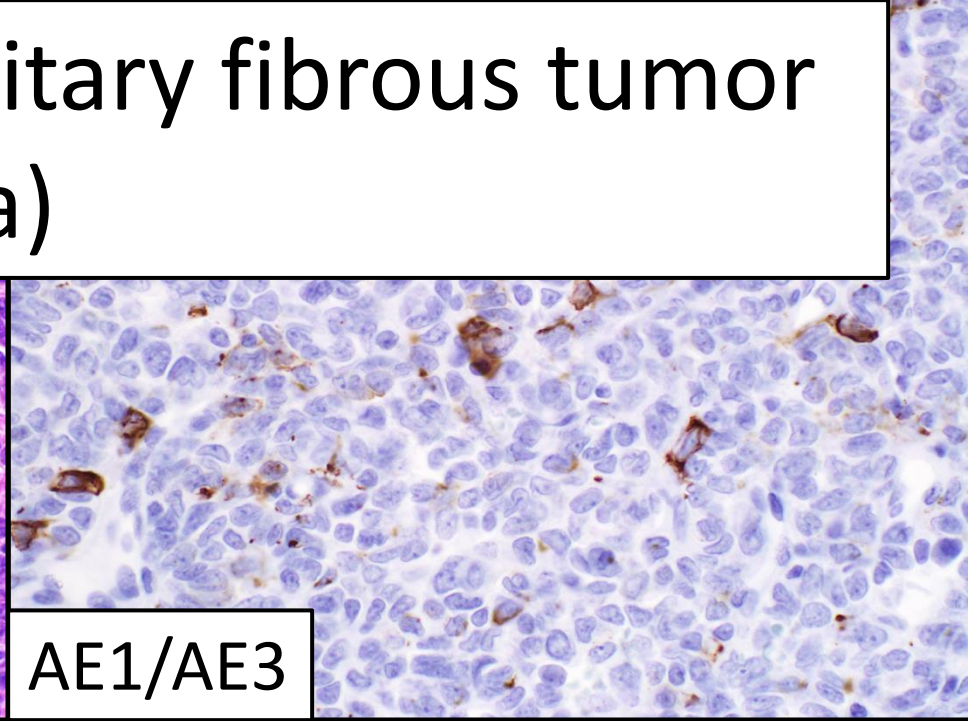


Doyle LA, et al. *Mod Pathol*. 2014 Mar;27(3)390-5.

Revised diagnosis: solitary fibrous tumor (hemangiopericytoma)

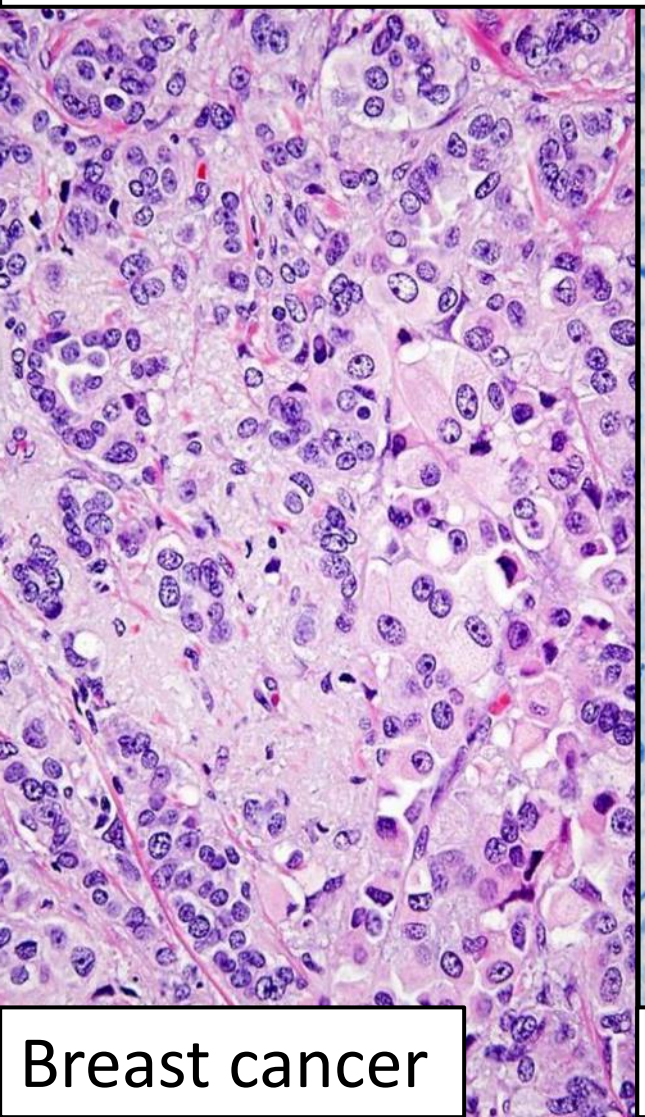


T1-T2 spinal lesion

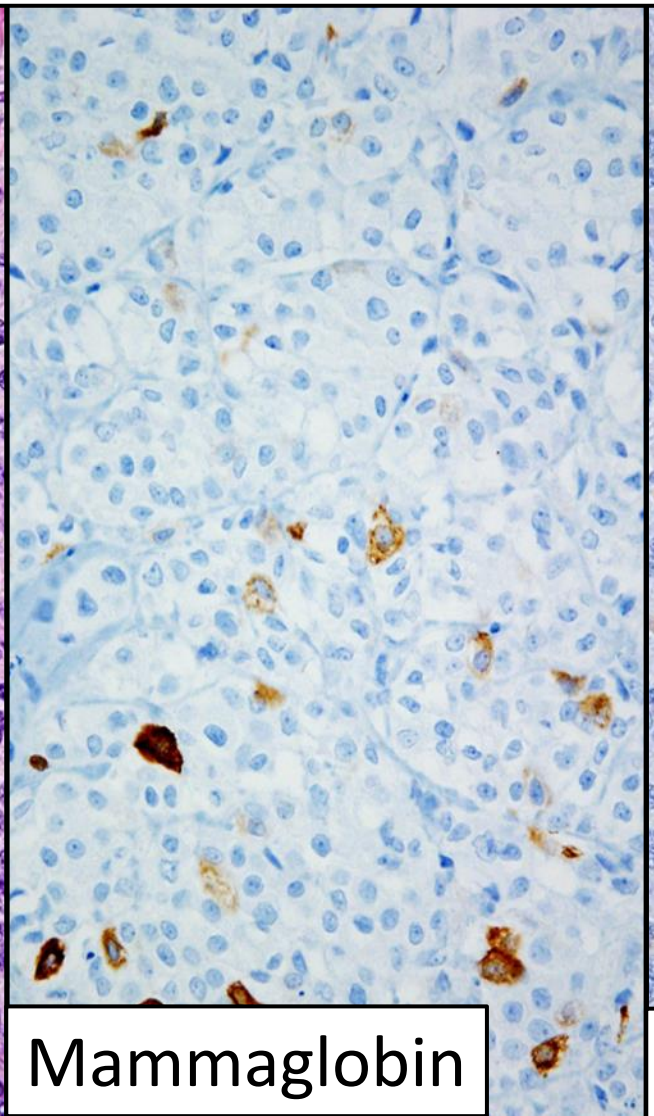


AE1/AE3

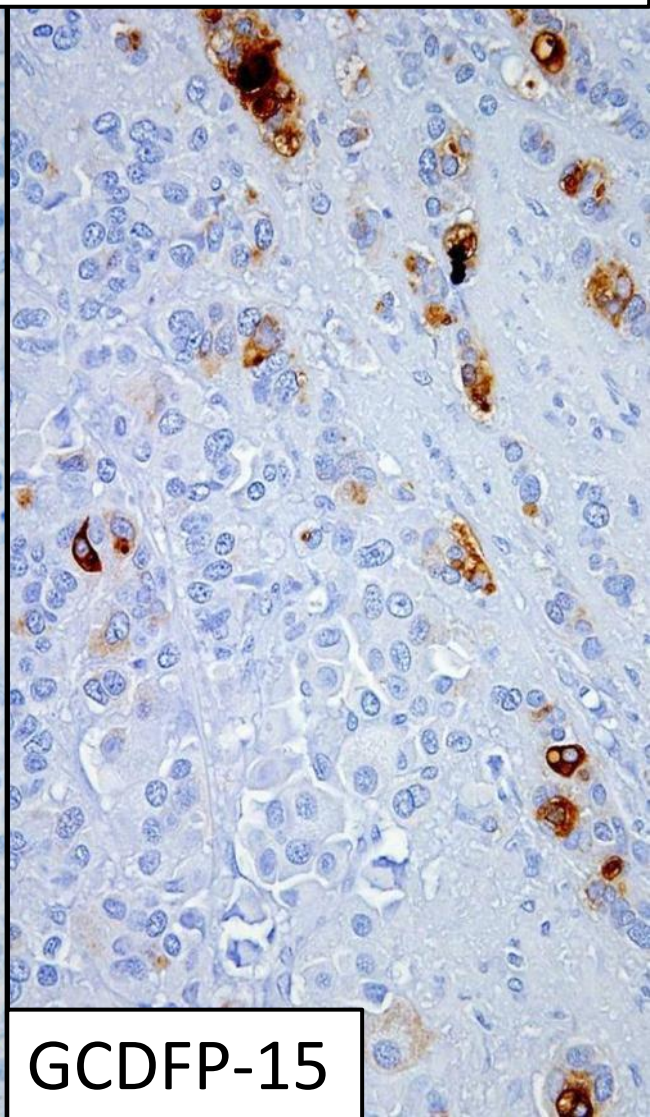
Historically, diagnostic armamentarium geared toward cytoplasmic or membranous differentiation markers; reduced sensitivity in poorly differentiated tumors



Breast cancer

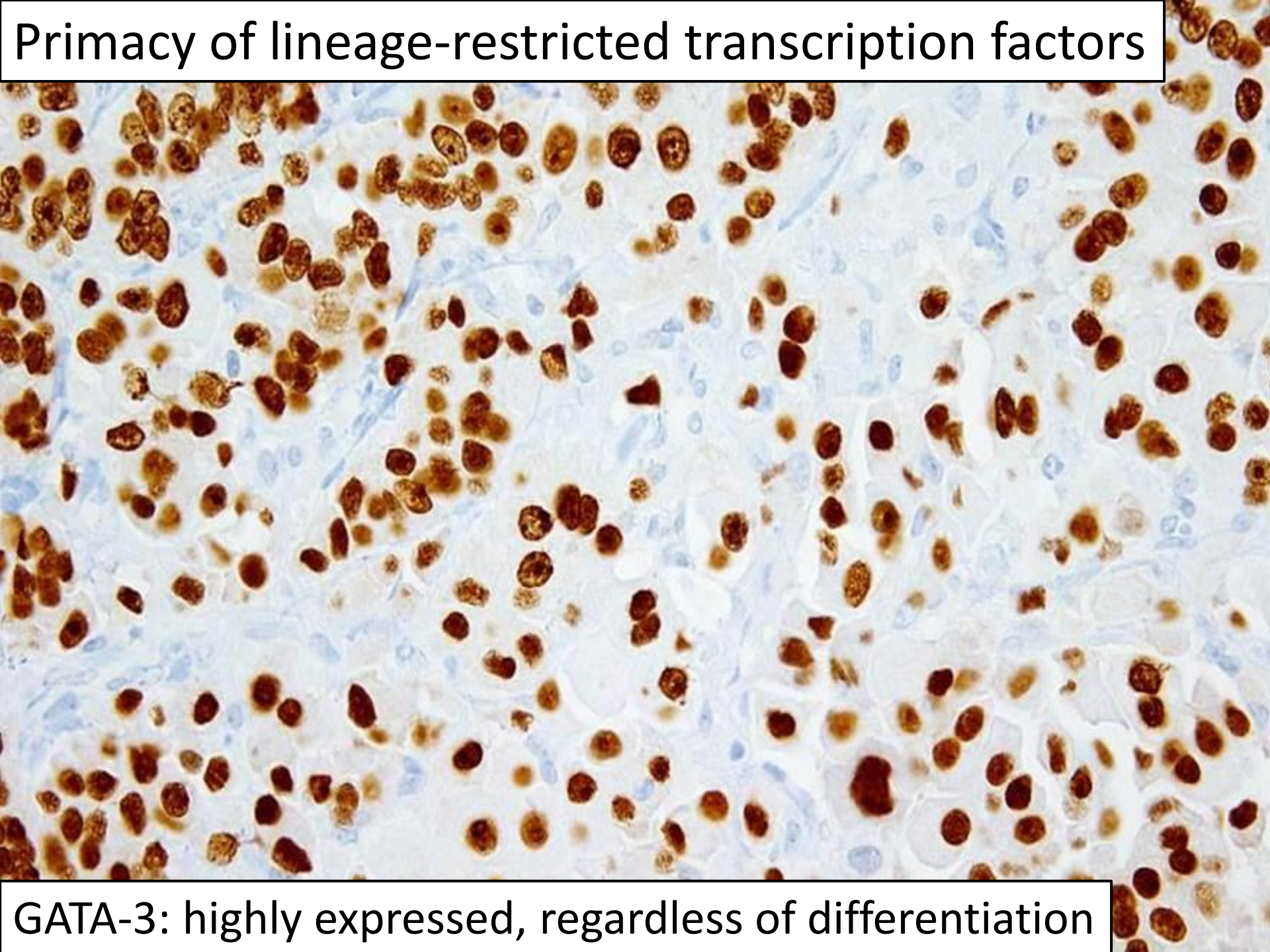


Mammaglobin



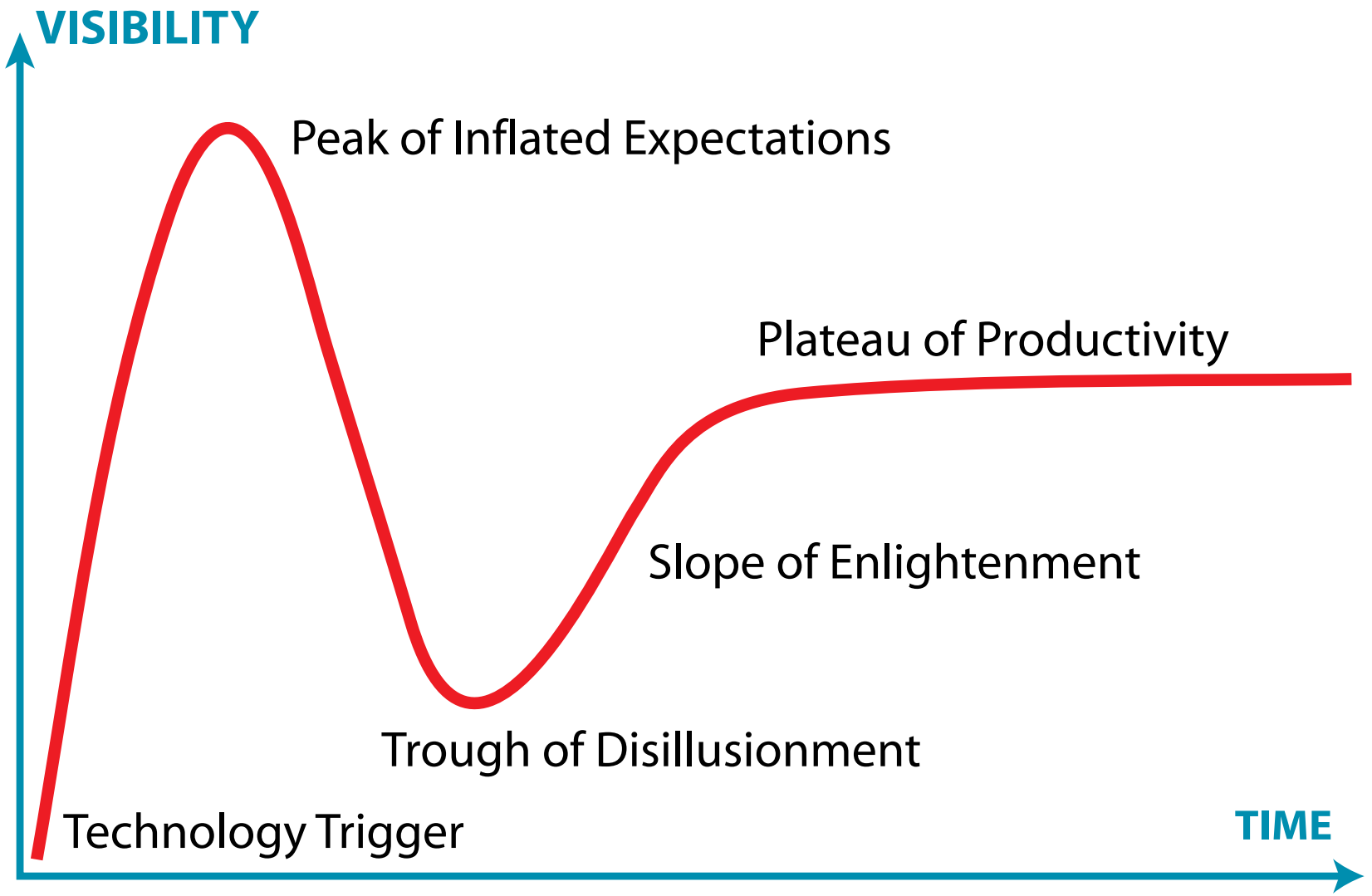
GCDFP-15

Primacy of lineage-restricted transcription factors



GATA-3: highly expressed, regardless of differentiation

Gartner Hype Cycle



ORIGINAL ARTICLE

Nuclear Brachyury Expression Is Consistent in Chordoma, Common in Germ Cell Tumors and Small Cell Carcinomas, and Rare in Other Carcinomas and Sarcomas

An Immunohistochemical Study of 5229 Cases

Markku Miettinen, MD, Zengfeng Wang, PhD,* Jerzy Lasota, MD,* Christopher Heery, MD,† Jeffrey Schlom, PhD,† and Claudia Palena, PhD†*

ORIGINAL ARTICLE

Sox10—A Marker for Not Only Schwannian and Melanocytic Neoplasms But Also Myoepithelial Cell Tumors of Soft Tissue

A Systematic Analysis of 5134 Tumors

Markku Miettinen, MD, Peter A. McCue, MD,† Maarit Sarlomo-Rikala, MD,‡ Wojciech Biernat, MD,§ Piotr Czapiewski, MD,§ Janusz Kopczynski, MD,‖ Lester D. Thompson, MD,¶ Jerzy Lasota, MD,* Zengfeng Wang, PhD,* and John F. Fetsch, MD#*

ORIGINAL ARTICLE

SALL4 Expression in Germ Cell and Non–Germ Cell Tumors

A Systematic Immunohistochemical Study of 3215 Cases

Markku Miettinen, MD, Zengfeng Wang, PhD,* Peter A. McCue, MD,† Maarit Sarlomo-Rikala, MD,‡ Janusz Rys, MD,§ Wojciech Biernat, MD,‖ Jerzy Lasota, MD,* and Yi-Shan Lee, MD**

ORIGINAL ARTICLE

GATA3: A Multispecific But Potentially Useful Marker in Surgical Pathology

A Systematic Analysis of 2500 Epithelial and Nonepithelial Tumors

Markku Miettinen, MD, Peter A. McCue, MD,† Maarit Sarlomo-Rikala, MD,‡ Janusz Rys, MD,§ Piotr Czapiewski, MD,‖ Krzysztof Wazny, MD,‖ Renata Langfort, MD,¶ Piotr Waloszczyk, MD,# Wojciech Biernat, MD,‖ Jerzy Lasota, MD,* and Zengfeng Wang, MD, PhD**

Abstract: GATA3 is a transcription factor important in the differentiation of breast epithelia, urothelia, and subsets of T lymphocytes. It has been suggested to be useful in the evaluation of carcinomas of mammary or urothelial origin or metastatic carcinomas, but its distribution in normal and neoplastic tissues

mors (12%). Common positivity was found in skin adnexal tumors (100%), mesothelioma (58%), salivary gland (43%), and pancreatic (37%) ductal carcinomas, whereas frequency of expression in adenocarcinomas of lung, stomach, colon, endometrium, ovary, and prostate was < 10%. Chromophobe renal cell carcinoma was a unique renal tumor with frequent positivity (51%), whereas oncocytomas were positive in 17% of cases but other types only rarely. Among mesenchymal and neuroectodermal tumors, paragangliomas were usually positive, which sets these tumors apart from epithelial neuroendocrine tumors. Mesenchymal tumors were only sporadically positive, except epithelia of biphasic synovial sarcomas. GATA3 is a useful marker in the characterization of not only mammary and urothelial but also renal and germ cell tumors, mesotheliomas, and paragangliomas. The multiple specificities of GATA3 should be taken into account when using this marker to detect metastatic mammary or urothelial carcinomas.

Key Words: GATA3, breast cancer, urothelial cancer, choriocarcinoma, endodermal sinus tumor, chromophobe renal carcinoma, mesothelioma, immunohistochemistry

(*Am J Surg Pathol* 2014;38:13–22)

GATA3-binding protein, commonly and from here on abbreviated as GATA3, is a transcription factor of the GATA family. These nuclear proteins recognize G-A-T-A nucleotide sequences in target gene promoters and activate or repress those genes.[1,2] GATA3 function is known to be important in the regulation of genes such as *MUC1*/*EMA* involved in the luminal differentiation of breast epithelium[3] and genes related to T-cell development.[4,5] Other known functions include gene regulation in the development or maintenance of skin, especially hair shafts,[6,7] trophoblast,[8–10] and some endothelial cells, especially in the great vessels.[11] Constitutional allelic loss of GATA3 (haploinsufficiency) causes a syndrome characterized by hypoparathyroidism, sensoneurial deafness, and renal malformations, abbreviated as HDR on the basis of these manifestations.[12]

GATA3 has been thus far explored in surgical pathology as a marker for breast and urothelial carcinomas. Most primary and metastatic mammary carcinomas

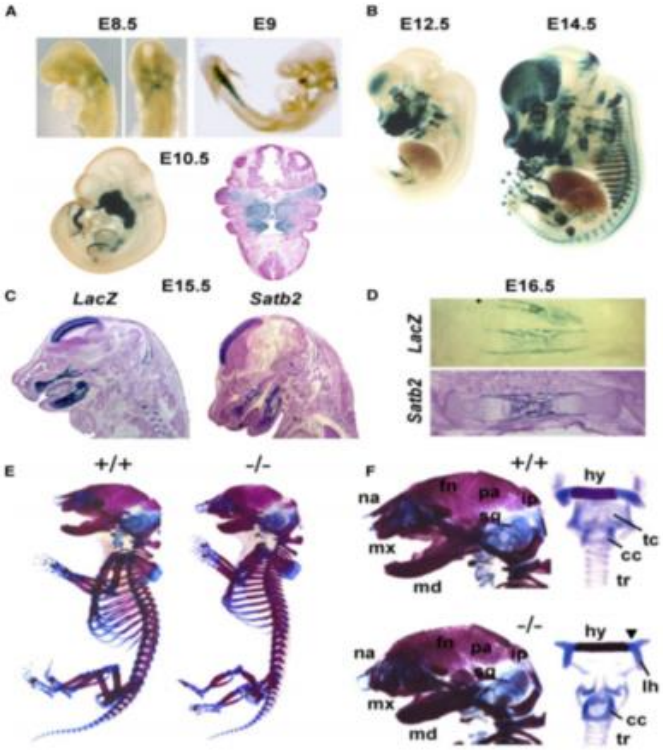
Stains I Love: Oligospecific Transcription Factors

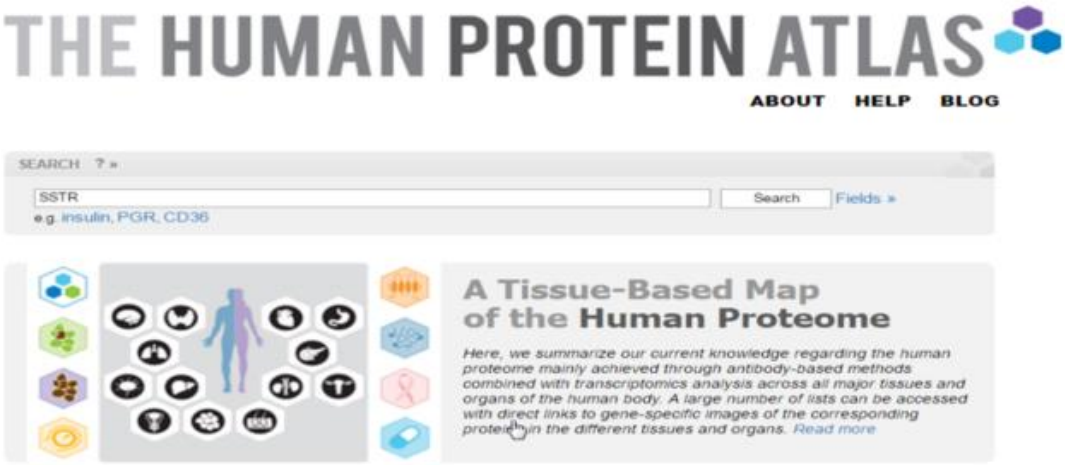
SATB2

SATB2 in Combination With Cytokeratin 20 Identifies Over 95% of all Colorectal Carcinomas

Kristina Magnusson, MSc, Meike de Wit, MSc,† Donal J. Brennan, MD, PhD,‡ Louis B. Johnson, MD, PhD,§ Sharon F. McGee, PhD,‡ Emma Lundberg, PhD,‖ Kirsha Naicker, MSc,‡ Rut Klinger, MSc,‡ Caroline Kampf, PhD,* Anna Asplund, PhD,* Kenneth Wester, PhD,* Marcus Gry, PhD,*‖ Anders Bjartell, MD, PhD,§¶ William M. Gallagher, PhD,‡ Elton Rexhepaj, PhD,‡ Sami Kilpinen, PhD,#** Olli-Pekka Kallioniemi, MD, PhD,# Eric Belt, MD,† Jeroen Goos, MSc,† Gerrit Meijer, MD, PhD,† Helgi Birgisson, MD, PhD,†† Bengt Glimelius, MD, PhD,‡‡§§ Carl A.K. Borrebaeck, PhD,‖‖ Sanjay Navani, MD,¶¶ Mathias Uhlén, PhD,‖ Darran P. O'Connor, PhD,‡ Karin Jirström, MD, PhD,## and Fredrik Pontén, MD, PhD**

Am J Surg Pathol • Volume 35, Number 7, July 2011



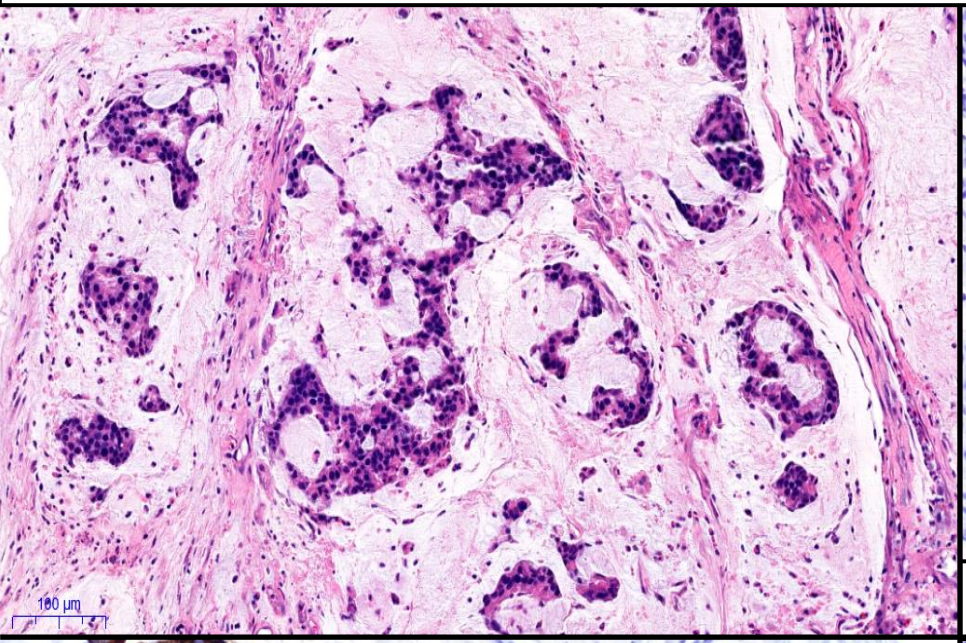


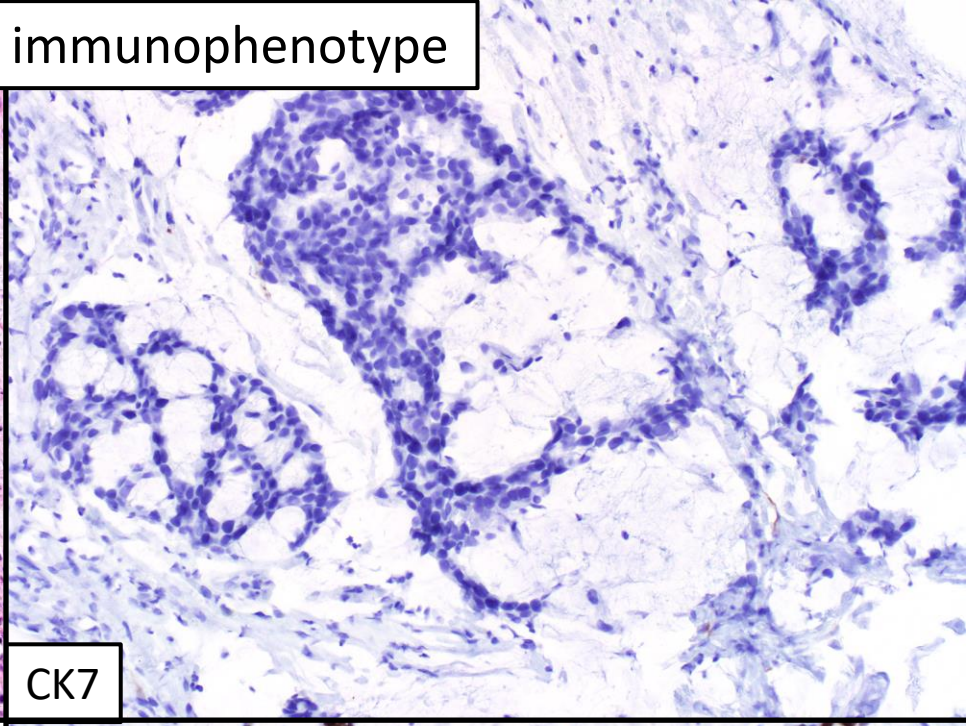
SATB2 Is a Multifunctional Determinant of Craniofacial Patterning and Osteoblast Differentiation

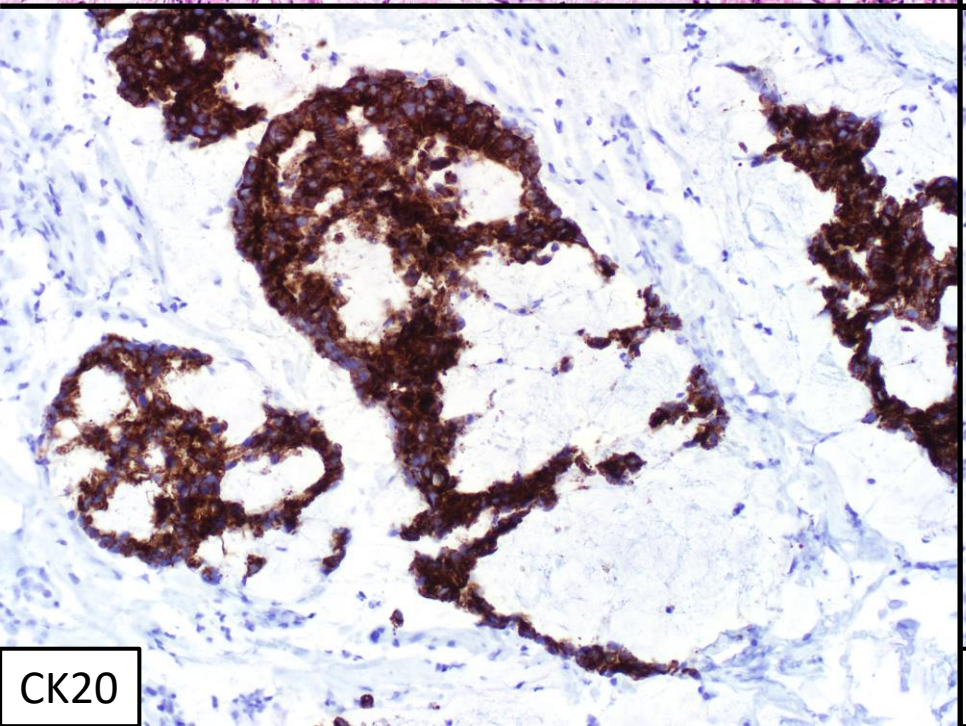
Gergana Dobreva,[1] Maria Chahrour,[2] Marcel Dautzenberg,[1] Laura Chirivella,[3] Benoit Kanzler,[1] Isabel Fariñas,[3] Gerard Karsenty,[2] and Rudolf Grosschedl[1,*]
[1]Max-Planck Institute of Immunobiology, Department of Cellular and Molecular Immunology, 79108 Freiburg, Germany
[2]Baylor College of Medicine, Department of Molecular and Human Genetics, Houston, TX 77030, USA
[3]Departamento de Biologia Celular, Unidad Mixta CIPF-UVEG, Universitad de Valencia, 46100 Burjasot, Spain

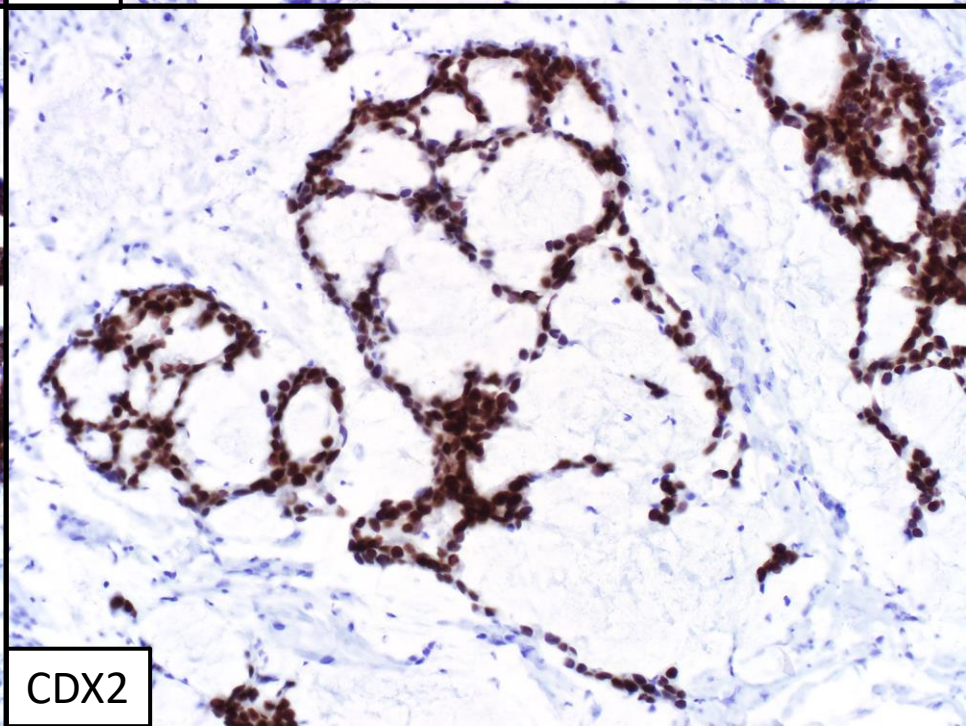
Cell *125*, 971–986, June 2, 2006 ©2006 Elsevier Inc.

Mucinous adenocarcinoma with lower GI immunophenotype

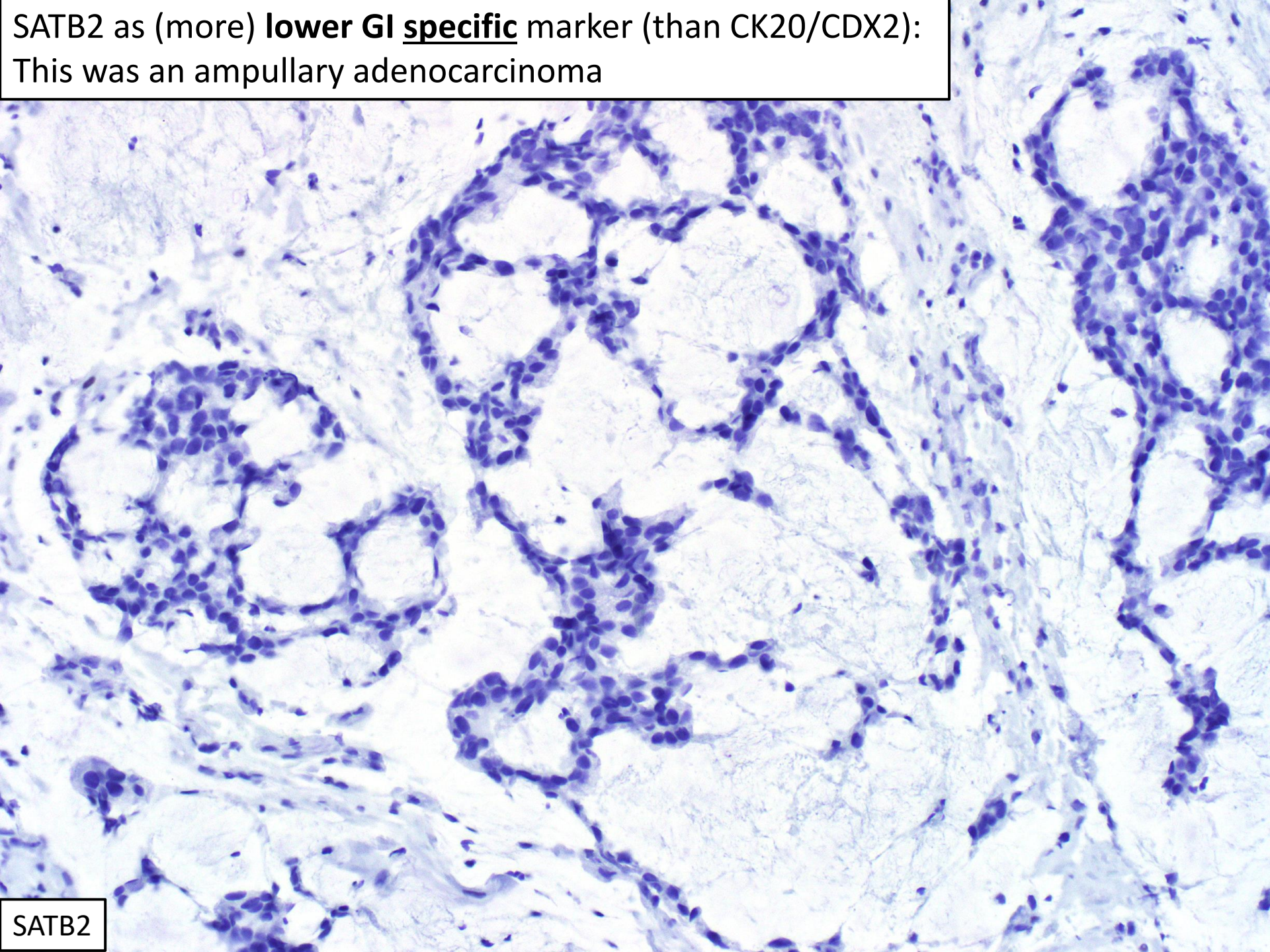




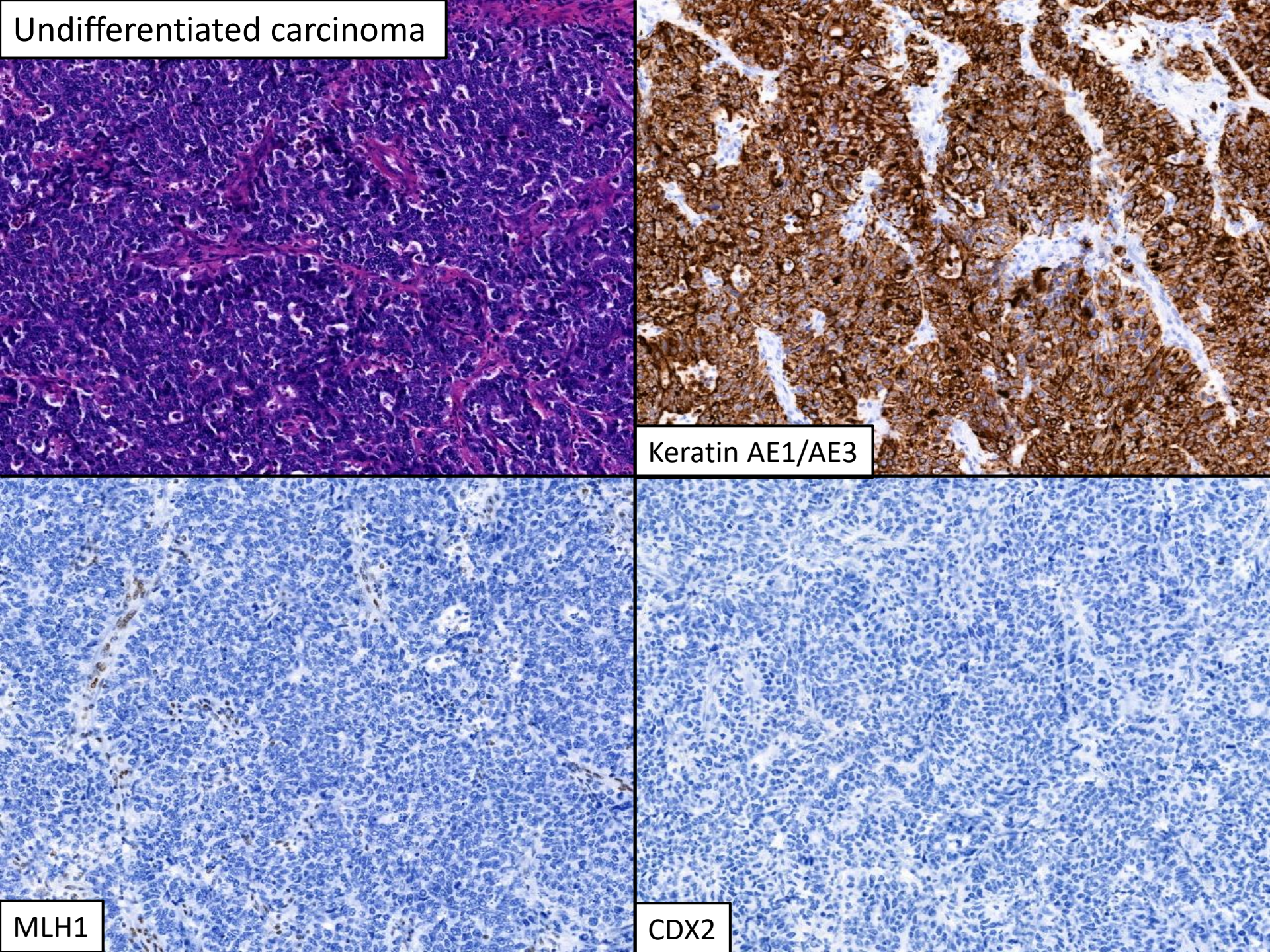




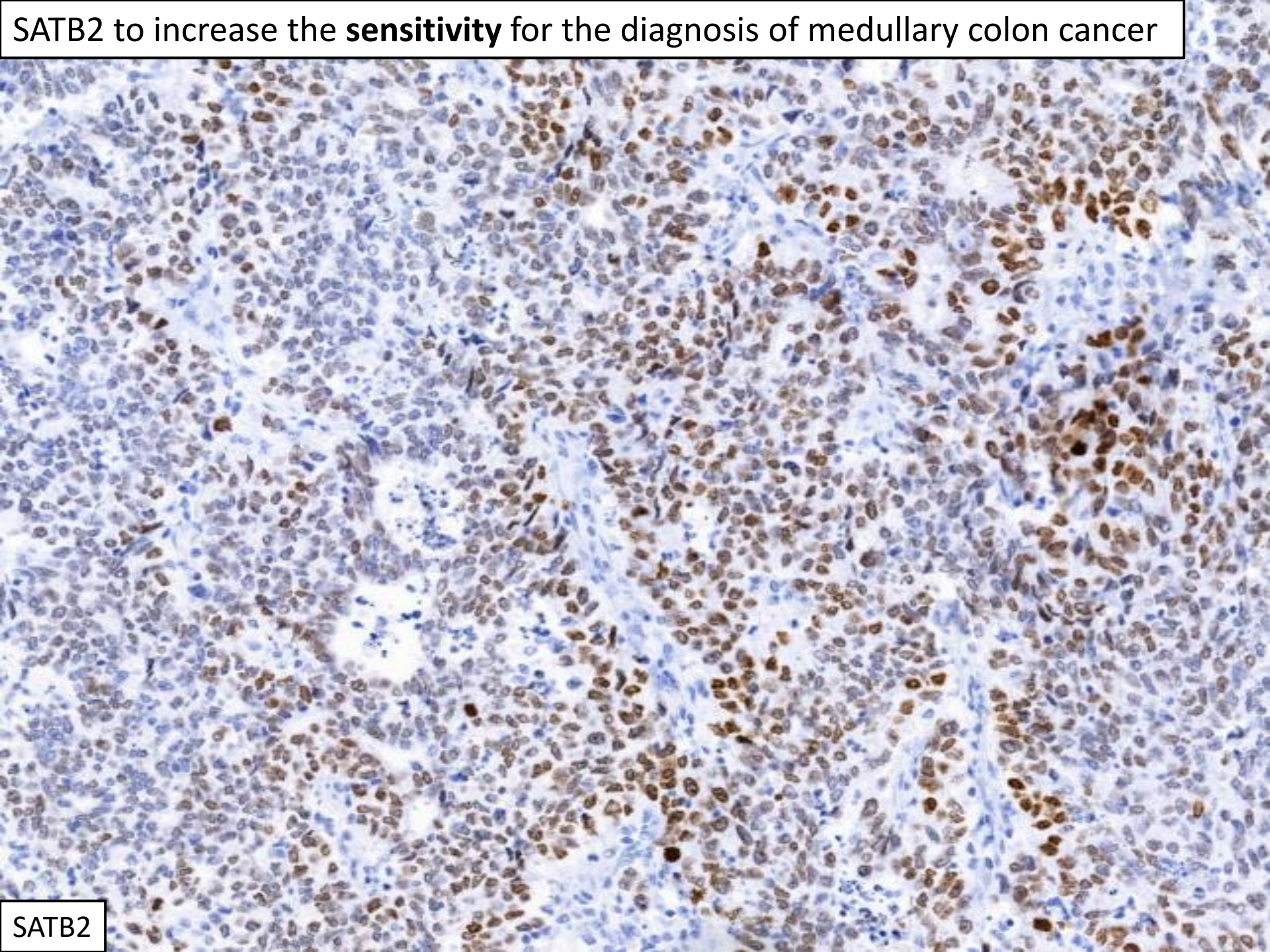
SATB2 as (more) **lower GI <u>specific</u>** marker (than CK20/CDX2):
This was an ampullary adenocarcinoma



SATB2

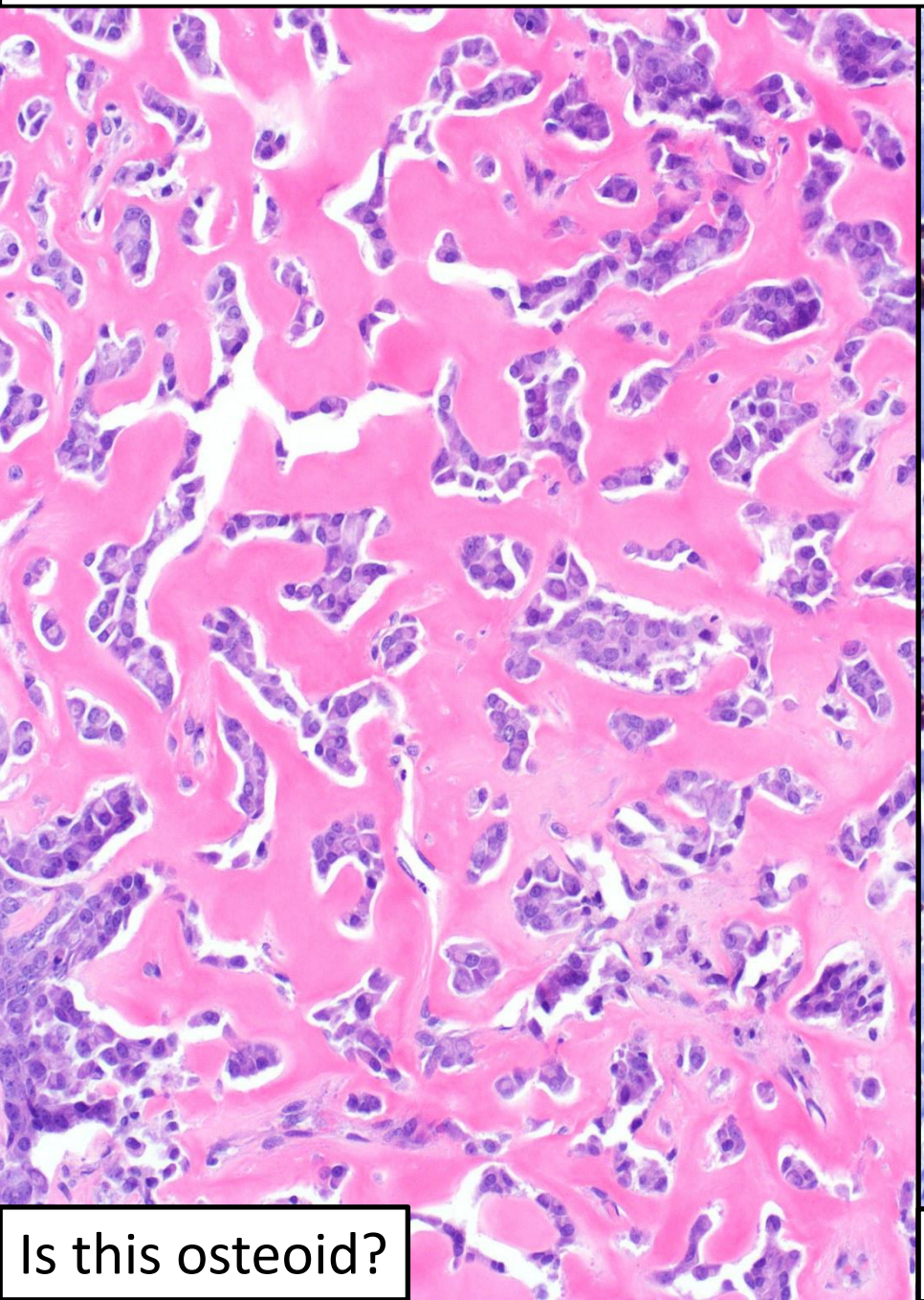

Undifferentiated carcinoma
Keratin AE1/AE3
MLH1
CDX2

SATB2 to increase the **sensitivity** for the diagnosis of medullary colon cancer

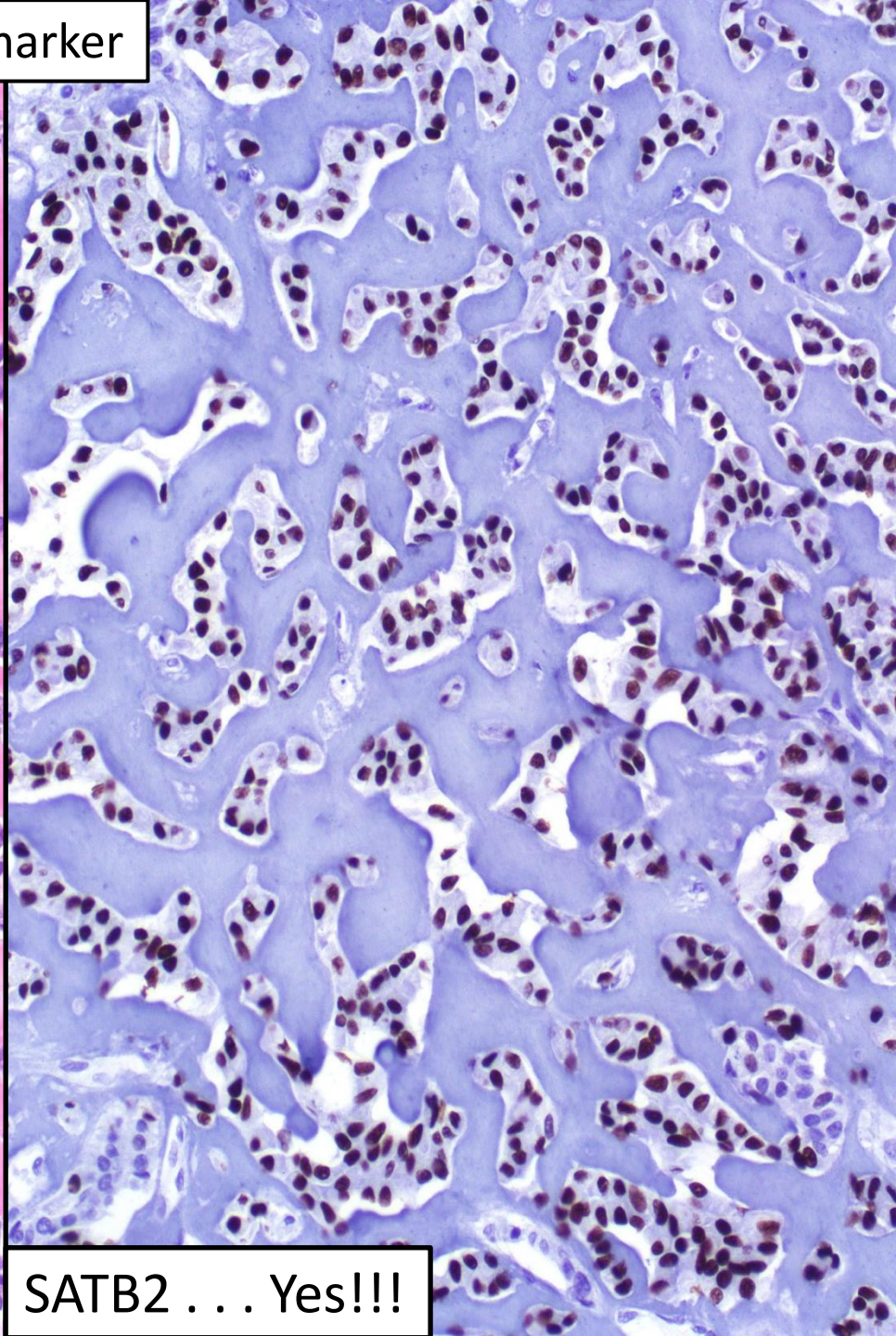


SATB2

SATB2 as an osteoblastic differentiation marker

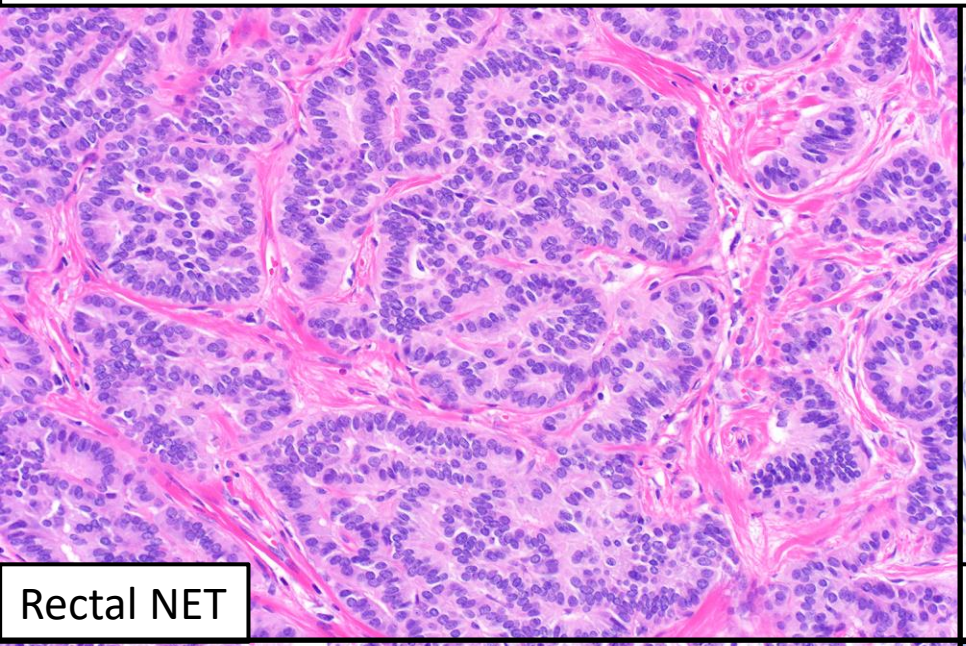


Is this osteoid?

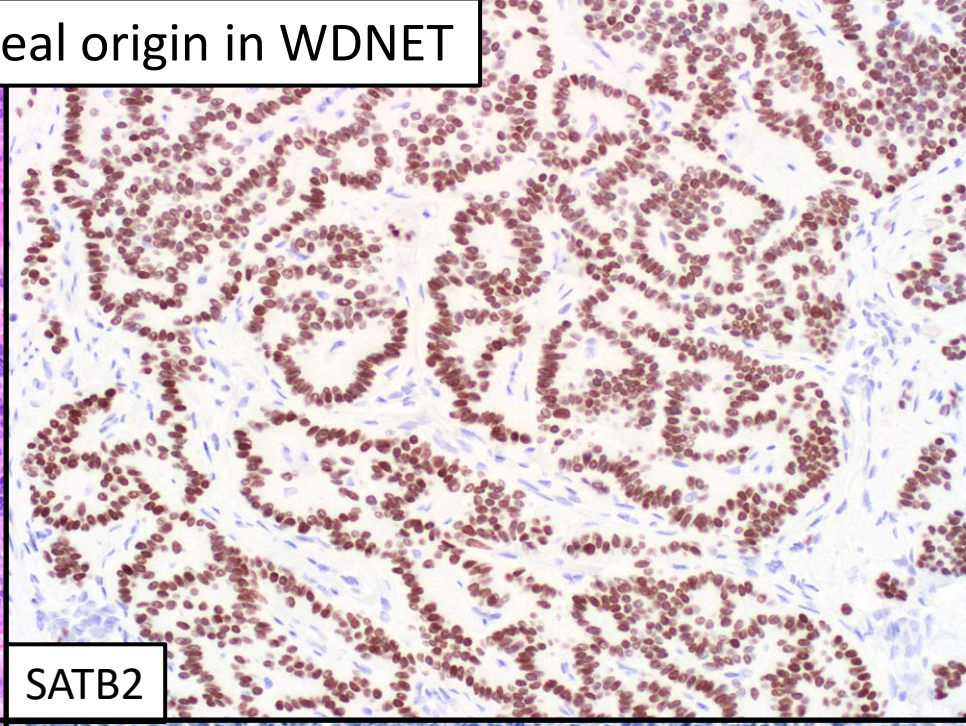


SATB2 . . . Yes!!!

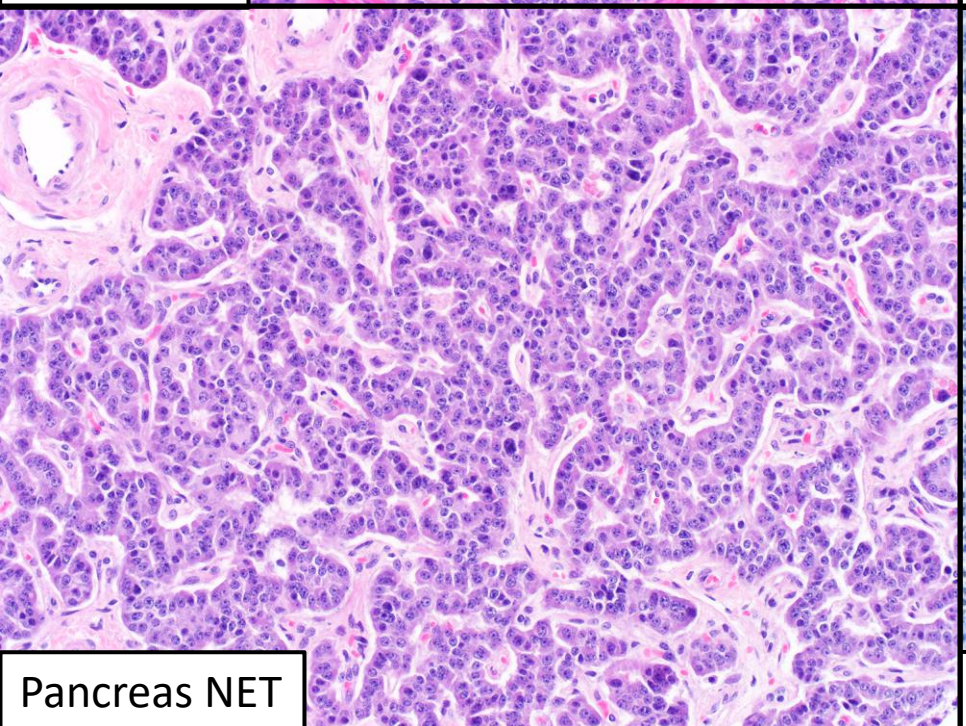
SATB2 as a marker of rectal and appendiceal origin in WDNET



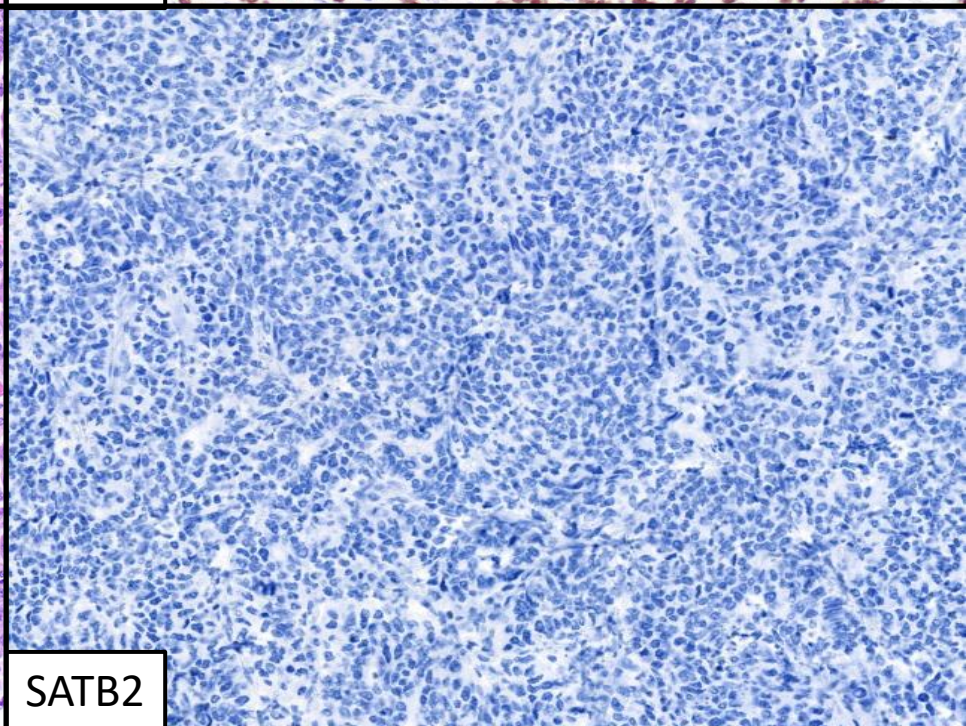
Rectal NET



SATB2



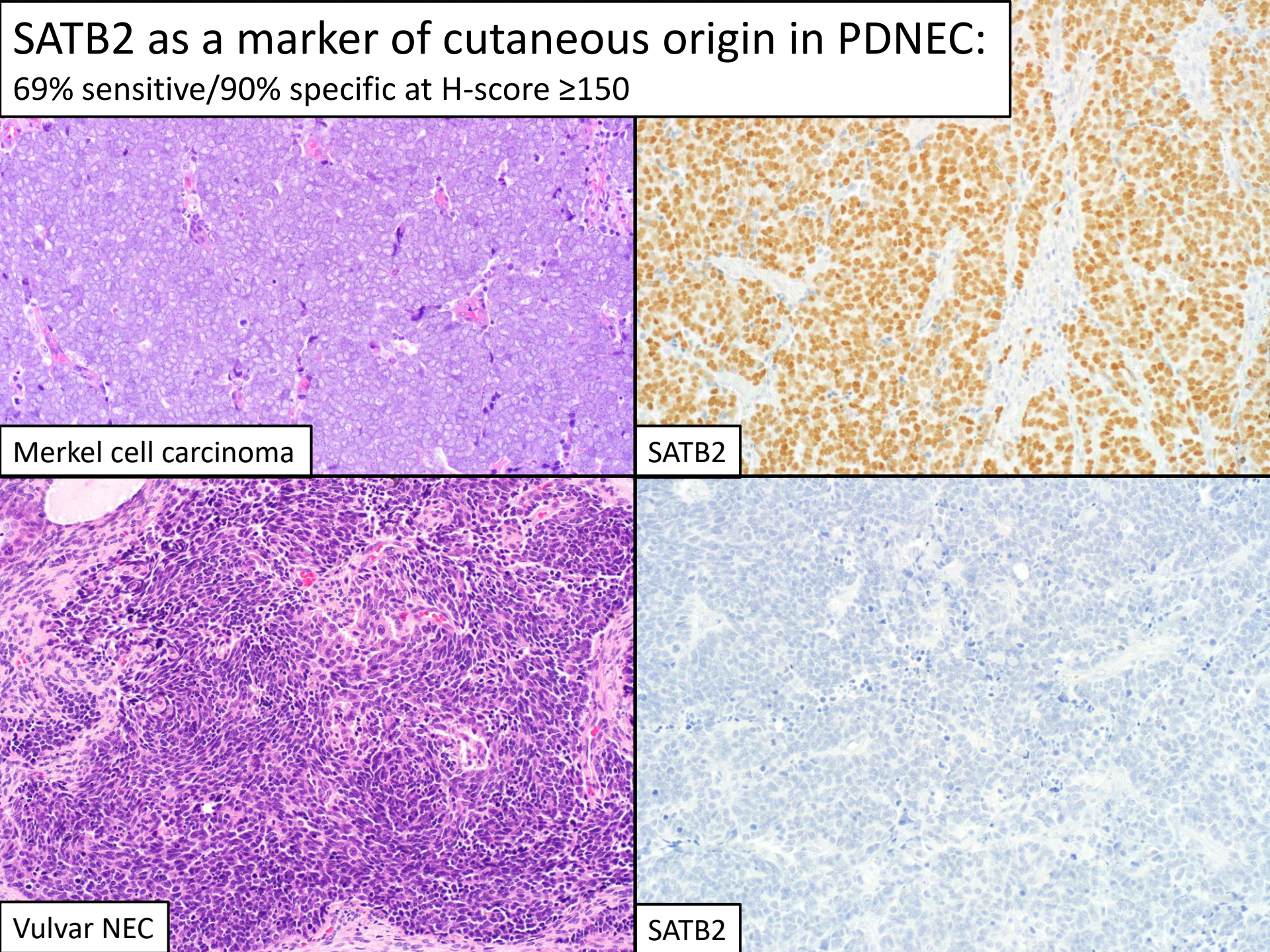
Pancreas NET



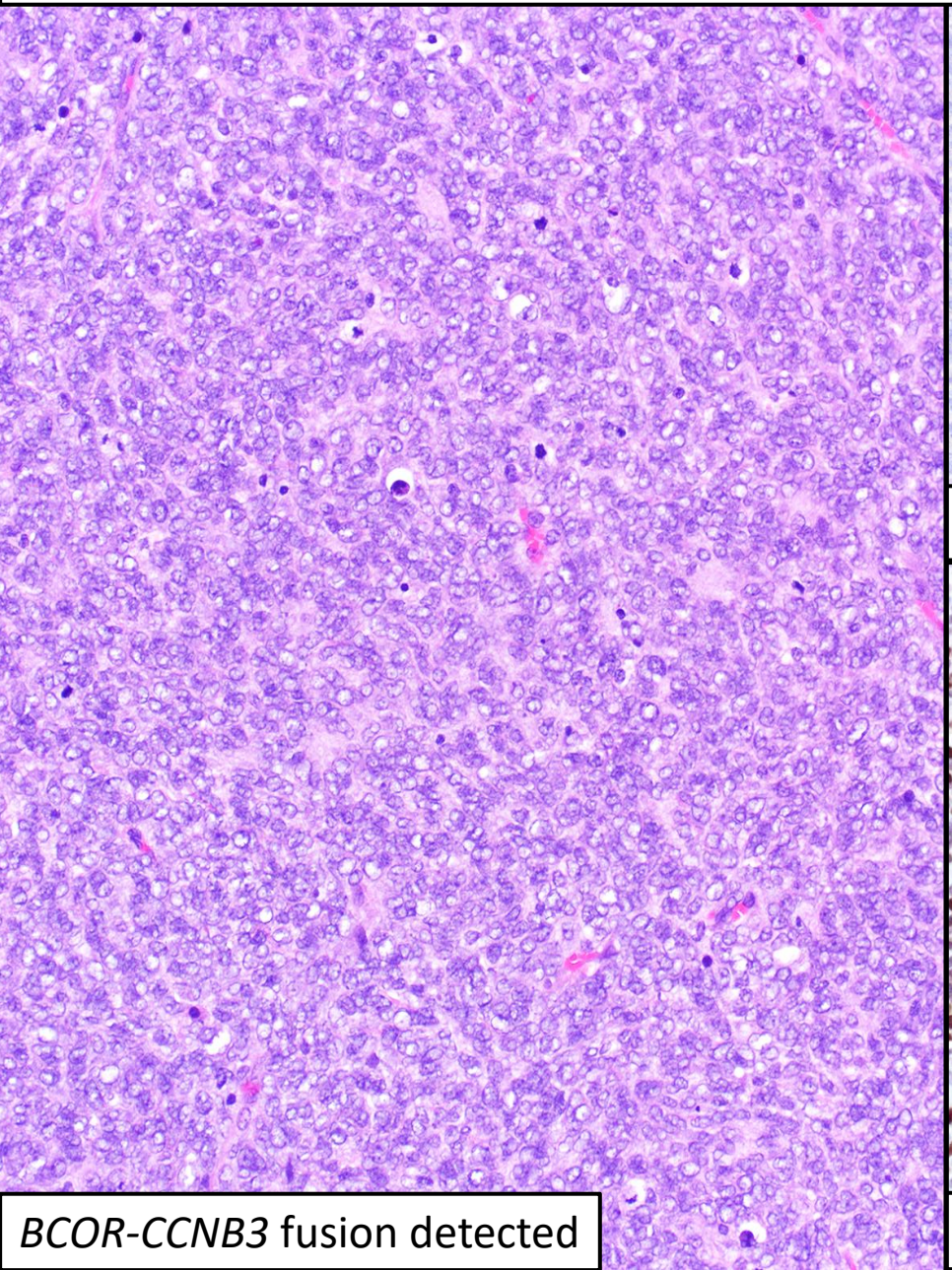
SATB2

SATB2 as a marker of cutaneous origin in PDNEC:

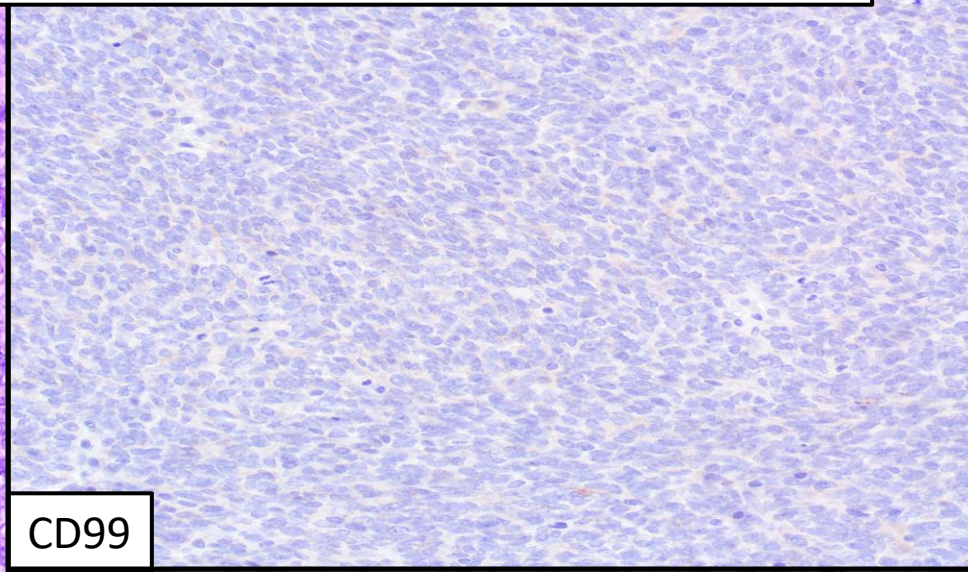
69% sensitive/90% specific at H-score ≥150



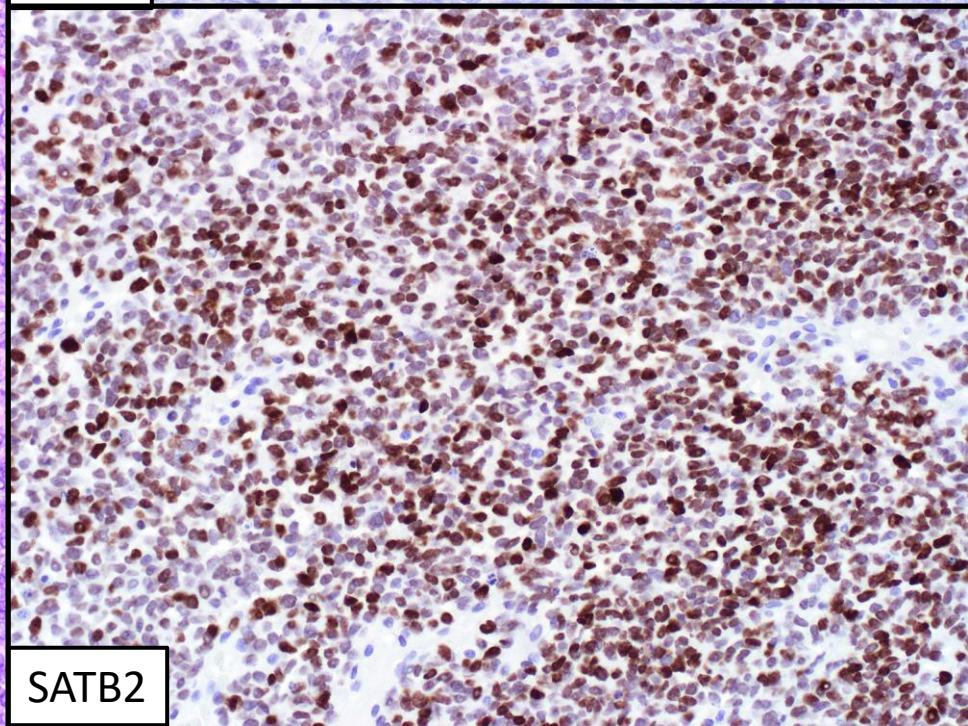
SATB2 as a marker of *BCOR*-rearranged sarcoma (and small cell osteosarcoma) in small round blue cell tumor differential



BCOR-CCNB3 fusion detected



CD99



SATB2

SATB2: Will it Swiss Army? Yes

- Lower GI adenocarcinoma (specific)
- Undifferentiated GI carcinoma (sensitive)
- Osteoblastic differentiation
- Rectal/appendiceal NET marker
- Merkel cell carcinoma
- *BCOR*-rearranged sarcoma

Dr. Bellizzi, Quick question for you. We have finally convinced our hospital to bring our IHC lab into the 21st century. We are adding GATA3, PAX8, SOX10 and ERG to our test menu to the delight of the pathologists in my group. Are there any particular clones of those antibodies you would recommend for a general pathology practice? We have Ventana instrumentation. Thanks! —Frank

May 30, 2020, 3:23 PM

Recommended protocols - GATA3

Search:

Epitope	Staining Platform	Clone name	Clone format	Version date	View
GATA3	Dako Autostainer Link 48 +	L50-823	CONC	14 Sep 2015	PDF
GATA3	Dako Autostainer Link 48 +	L50-823	CONC	04 Sep 2018	PDF
GATA3	Dako Omnis	L50-823	CONC	13 Sep 2015	PDF
GATA3	Dako Omnis	EP368	CONC	09 Oct 2018	PDF
GATA3	Dako Omnis	L50-823	CONC	09 Aug 2018	PDF
GATA3	Leica BOND III	L50-823	CONC	11 Sep 2015	PDF
GATA3	Leica BOND III	L50-823	CONC	30 Jul 2018	PDF
GATA3	Ventana Benchmark Ultra	L50-823	CONC	15 Sep 2015	PDF
GATA3	Ventana Benchmark Ultra	L50-823	RTU	06 Aug 2018	PDF
GATA3	Ventana Benchmark Ultra	L50-823	CONC	22 Aug 2018	PDF

Frank, please call me Andrew!!! Four of my favorites. Antibody (clone) performance DOES vary for some antibodies depending on platform. I run Dako Autostainer Link 48's. For these 4 I run--GATA-3: L50-823 (Cell Marque), PAX8: EP298 (BioSB), SOX10: EP268 (Epitomics), ERG: EPR3864 (abcam). These are actually 4 good "case stories." For GATA-3 we used to run HG3-31 before switching for L50-823 from another vendor (who discontinued it). For PAX8 I switched from polyclonal to monoclonal after someone misdiagnosed a DLBCL as a ccRCC because polyclonal PAX8 crossreacts with other PAX-family transcription factors, including PAX5. For SOX10 I also switched from a polyclonal to a monoclonal when a good one became available. For ERG . . . we're using the same clone that we validated 6 years ago (steady as she goes). For clone selection, "phoning a friend" works well, especially for more esoteric antibodies. I also rely heavily on the published literature. For workhorses like you're bringing up, the BEST place to go is the NordiQC website (it's the Scandinavian IHC proficiency testing "programme"). They go so far as to recommend specific protocols (combinations of platform, clone, retrieval, dilution, etc). nordiqc.org/recommended.php Stay in touch and Happy Staining, andrew

May 31, 2020, 12:29 PM ✓

Frank is looking for GATA-3, PAX8, SOX10, and ERG

Hi Andrew! Thanks again for your impressive handling of the SO awards this year, it seems like I have been flying by the seat of my pants all year between online learning, meetings, etc, but you made it look effortless. Inspirational! Quick unrelated question re: the IHC lab. Thermo-Fisher discontinued our toxoplasma ab (clone AB-1) and I am not sure where to turn to find a good source for a (relatively) esoteric antigen. I think Leica used to sell the Novocastra TP3 clone, which seems reasonable to try (we run Ventanas w/Ultraview), and nothing jumped out at me on Biocompare. What clone/supplier do you use, and do you have other recommendations for how to search for commonly used clones for rare-ish antigens besides biocompare, pubmed, and nordiqc? Thank you!!!! PS Sorry about the Hawkeyes, who could have guessed the Ducks would shoot 80% (est.) from the floor ?!

Mar 23, 2021, 7:25 PM

Thanks for your kind words. I use word of mouth 1st (I'm close with Jason Hornick's lead tech, Mei), NordiQC 2nd, pubmed third, and Biocompare 4th. Do you know David Rimm??? He's a "lab guy" but his lab centers on quantitative IHC/IF of predictive markers. He's on my CAP Committee and I have been reading his stuff. HE uses Antibodypedia and BenchiSci . . . I have not checked these out but mean to the next time I take a flier on a new marker. Otherwise, specifically for ID IHC . . . Sherif Zaki is where the buck stops . . . he is the Chief of ID Path at the CDC. Finally, I don't do this testing, but I'm happy to put out an APB to the IHC Committee to see if anyone does. Also . . . for rareish antigens . . . I'm happy to be your Mei:)

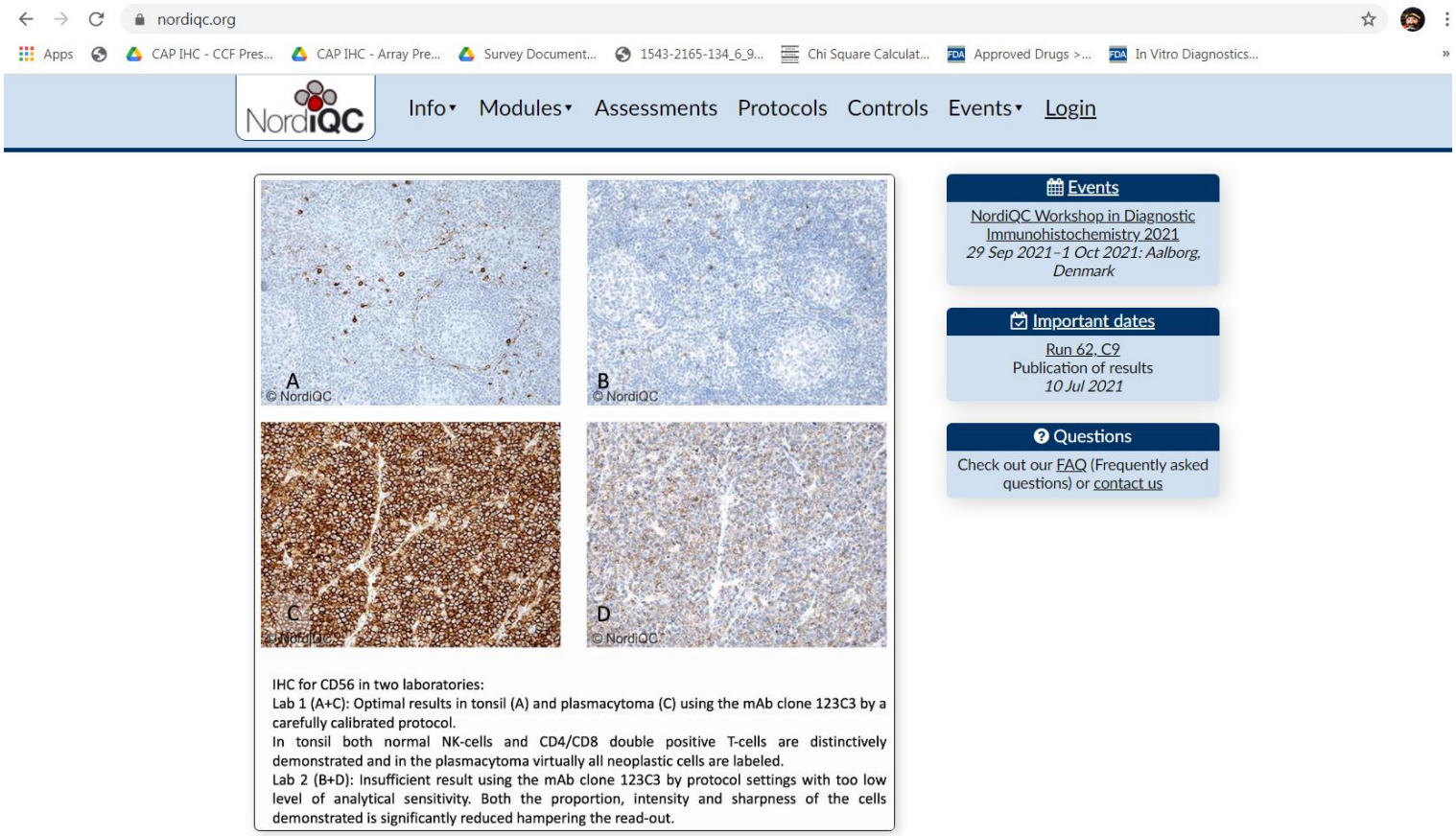
Mar 23, 2021, 7:56 PM ✓

Phil is looking for toxoplasma

Antibody Selection

- Phone a friend (esoteric antibodies)
- Published literature
- NordiQC (workhorses)
- Biocompare
- Antibodypedia
- BenchSci

NordiQC – Scandanavian IHC EQA



Results - Run 61, B31, H19

Detailed Platform Specific Protocols

NordiQC Info Modules Assessments Protocols Controls Events Login

Recommended protocols - HER2 IHC

Search:

Epitope	Staining Platform	Clone name	Clone format	Version date	View
HER2 IHC	Dako Autostainer Link 48 +	SP3	CONC	21 Aug 2019	PDF
HER2 IHC	Dako Autostainer Link 48 +	SP3	Other	30 Dec 2019	PDF
HER2 IHC	Dako Autostainer Link 48 +	HercepTest (polyclonal)	RTU	05 Jan 2021	PDF
HER2 IHC	Dako Autostainer Link 48 +	Polyclonal	CONC	10 Feb 2021	PDF
HER2 IHC	Dako Omnis	Polyclonal	CONC	10 Dec 2020	PDF
HER2 IHC	Dako Omnis	DG44	RTU	15 Apr 2021	PDF
HER2 IHC	Leica BOND III	Oracle (clone CB11)	RTU	11 Dec 2019	PDF
HER2 IHC	Leica BOND III	Polyclonal	CONC	30 Dec 2019	PDF
HER2 IHC	Leica BOND Max	SP3	CONC	27 Aug 2019	PDF
HER2 IHC	Ventana Benchmark Ultra	SP3	CONC	12 Aug 2019	PDF
HER2 IHC	Ventana Benchmark Ultra	SP3	Other	20 Dec 2019	PDF
HER2 IHC	Ventana Benchmark Ultra	PATHWAY (clone 4B5)	RTU	19 Feb 2021	PDF
HER2 IHC	Ventana Benchmark XT	Polyclonal	CONC	29 Dec 2020	PDF

Biocompare, Antibodypedia, BenchSci

BenchSci

Application: Immunohistochemistry | Target/Protein: NCOA2

Save Search | Guide to BenchSci

Filters

- Application

Figure Usage Data
- Organism Tested
- Tissue Used
- Cell Type Used
- Cell Line Used
- Disease

Supplier Filters
- Availability
- Company

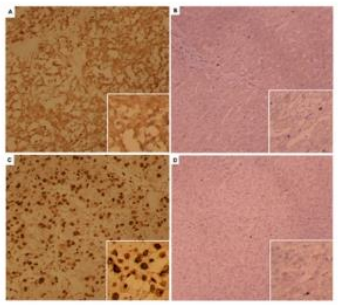
Antibody Specs
- Verification
- Reactivity

FIGURES (69) | PRODUCTS (168)

Published (16) | Supplier (53) | Third Party (0)

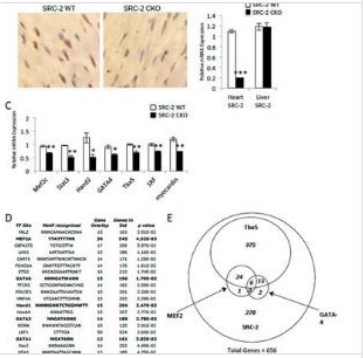
SORT BY: BEST MATCH

Published Figure
PLoS ONE 2011



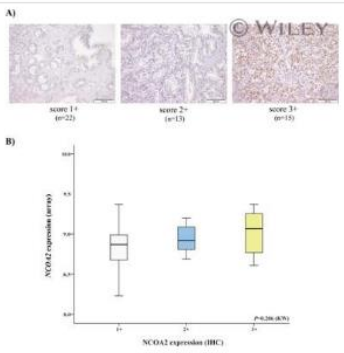
IHC | IF | +3

Published Figure
Journal of Biological Chemistry 2014



IHC | ChIP

Published Figure
Genes, Chromosomes and Can... 2016



IHC | IS

Published Figure
The Journal of Clinical Investig... 2014

Published Figure
PLoS ONE 2014

Published Figure
Scientific Reports 2017

Optimization

A Practical Approach for Evaluating New Antibodies in the Clinical Immunohistochemistry Laboratory

Eric D. Hsi, MD

• Paraffin section immunohistochemistry (IHC) is widely used in diagnostic surgical pathology. Today, it is difficult to imagine the practice of surgical pathology without IHC. The availability of automated immunostainers with reagent rental contracts makes this technology commonplace. One potential danger is that many laboratories are now offering immunostains without significant prior knowledge or experience in IHC. As part of its mission, the Cell Markers Committee of the College of American Pathologists offers this manuscript as a basic guide to introducing new antibodies in the clinical IHC laboratory. Issues relating to regulatory developments, antibody selection, staining optimization, and test validation are addressed.
(*Arch Pathol Lab Med.* 2001;125:289–294)

Paraffin section immunohistochemistry (IHC) has now become indispensable in the practice of surgical pathology. We routinely rely on IHC to assist us in the diagnosis and classification of neoplasms. A recent 4-month survey of cases accessioned in the Department of Anatomic Pathology at the Cleveland Clinic showed that approximately 13% of cases made use of IHC. No longer limited to large university-based or reference laboratories, IHC is also widespread in community hospitals. The reasons for this widespread use include the proliferation of automated immunostainers from many vendors, which makes IHC possible without prior technical expertise (thus not requiring significant increases in personnel), and the large variety of primary antibodies that recognize fixation-resistant epitopes.

Despite the widespread use of IHC, there is a surprising lack of standardization among IHC laboratories. Occasional articles have appeared detailing potential problems and putting forth proposals for improving the quality of stains or standardizing IHC.[1-3] A recent review showed that the governmental regulations pertaining to IHC primarily address general laboratory operations.[4] Such regulations require compliance with guidelines regarding standard laboratory manual formats; reagent labeling and storage practices; equipment maintenance; adequate record keeping; quality control programs (use of appropriate positive and negative controls); and quality assurance programs (such as participation in proficiency testing programs approved by the Clinical Laboratory Improvement Amendments '88).[5] There is little guidance or regulation concerning the desired diagnostic performance requirements of a particular stain. The National Committee for Clinical Laboratory Standards (NCCLS) consensus guidelines provide more details about both technical and theoretical aspects of IHC, and the reader is referred to this document for further information.[6]

A recent occurrence familiar to those who deal with IHC on a daily basis is the implementation of the Food and Drug Administration (FDA) ruling on the classification of immunohistochemical reagents and kits.[7] This ruling has led to the classification of the great majority of immunohistochemical reagents as "Analyte Specific Reagents" (ASR).[8,9] Under this ruling, most immunohistochemical staining reagents were reclassified as class I medical devices, exempting them from premarket notification. This classification is allowed because IHC staining results are incorporated into the diagnostic report by a pathologist as one part of the entire diagnostic evaluation. The IHC results are not stand-alone results. A few IHC stains, such as estrogen and progesterone receptor stains, are considered class II devices. These stains have no routine morphologic correlates but do have substantial and accepted scientific validation. Class III devices would include stains that are not considered part of the surgical pathology diagnostic process and would result in a stand-alone report to a physician. Such tests require premarket notification and specific FDA approval.

The FDA ruling essentially allows IHC laboratories to continue operating as they had been prior to the ruling. Manufacturers must label the reagents they sell for diagnostic use (but for which they have not sought FDA clearance) as ASRs. Manufacturers have the responsibility of following good manufacturing practices to ensure that antibodies are of consistent high quality and have the specificities that are claimed. However, a disclaimer is required in all surgical pathology reports in which IHC is used that states that the individual laboratory (not the manufacturer of reagents) has the ultimate responsibility for assuring the quality of staining.[10] Thus, the laboratory director has the duty to provide a high-quality immunostain and to document its performance.

With this as background, it is the intention of this article to guide the reader in what we feel is an appropriate and practical approach for evaluating new antibodies in the modern automated IHC laboratory, with brief theoretical optimal staining with the correct cellular localization must be developed.

Accepted for publication September 8, 2000.
From the Departments of Anatomic and Clinical Pathology, Cleveland Clinic Foundation (for the Cell Markers Resource Committee of the College of American Pathologists), Cleveland, Ohio.
Reprints: Eric D. Hsi, MD, Head, Section of Hematopathology, Department of Clinical Pathology, L-11 Cleveland Clinic Foundation, 9500 Euclid Ave, Cleveland, OH 44195 (e-mail: hsie@ccf.org).

Sample Staining Protocol Worksheet for Evaluating a New Antibody*

Antibody/Specificity: X Vendor: Company Catalog #: 327 Lot #: 1
Date: 4/1/00 Positive Control Tissue: Tonsil

Dilution	Antigen Retrieval	Primary Incubation Time, min	Detection System	Detection Amplification	Formalin-Fixed Tissue	B5-Fixed Tissue	Hollandes-Fixed Tissue
1:10	10 mmol/L citrate, pH 6 20 min	32	ABC, DAB	No	4+	3+	2+
1:20	10 mmol/L citrate, pH 6 20 min	32	ABC, DAB	No	4+	3+	2+
1:40	10 mmol/L citrate, pH 6 20 min	32	ABC, DAB	No	4+	3+	1+
1:80	10 mmol/L citrate, pH 6 20 min	32	ABC, DAB	No	3+	2+	0
1:160	10 mmol/L citrate, pH 6 20 min	32	ABC, DAB	No	2+	2+	0
1:10	1 mmol/L EDTA pH 8, 20 min	32	ABC, DAB	No	4+	4+	3+
1:20	1 mmol/L EDTA pH 8, 20 min	32	ABC, DAB	No	4+	4+	3+
1:40	1 mmol/L EDTA pH 8, 20 min	32	ABC, DAB	No	4+	4+	3+
1:80	1 mmol/L EDTA pH 8, 20 min	32	ABC, DAB	No	4+	4+	2+
1:160	1 mmol/L EDTA pH 8, 20 min	32	ABC, DAB	No	3+	3+	±
1:10	Protease, 8 min	32	ABC, DAB	No	2+	2+	1+
1:20	Protease, 8 min	32	ABC, DAB	No	2+	1+	1+
1:40	Protease, 8 min	32	ABC, DAB	No	1+	0+	±
1:80	Protease, 8 min	32	ABC, DAB	No	0	0+	0
1:160	Protease, 8 min	32	ABC, DAB	No	0	3+	0

* Sample worksheet for the optimization of a monoclonal antibody with a recommended working dilution of 1:40 demonstrating optimal reactivity at 1:40 using EDTA-based heat-induced epitope retrieval. These conditions could then be used on a validation series of cases mimicking the intended diagnostic situation for which the antibody will be used. Scoring is based on the intensity of staining of the appropriate cellular elements with proper subcellular localization, as well as the proportion of cells expected to be immunoreactive, on a scale of 0–4+. 0 indicates negative; 1+, weakly positive in a small percentage of the cells expected to be positive; 2+, weakly positive in the majority of the cells expected to be positive; 3+, moderately positive in the majority of cells expected to be positive; and 4+, strongly positive in all cells expected to be positive. ABC indicates avidin-biotin complex; DAB, 3,3′-diaminobenzidine.

With the increased sensitivity for detecting antigens comes the increased ability to detect a false signal due to nonspecific staining. In particular, it has been shown that endogenous biotin activity can be retrieved by HIER, causing false-positive, granular cytoplasmic reactions.[35] This phenomenon was responsible for the erroneous report of inhibin staining in hepatocellular carcinomas that was subsequently shown to be due to endogenous biotin.[36,37] This source of false-positive staining can be eliminated by adding a biotin-blocking step.[35,38] Other potential causes of false-positive staining include endogenous peroxide in peroxidase-based systems and endogenous phosphatase in alkaline phosphatase–based systems. Both of these sources can be minimized by blocking with hydrogen peroxide and levamisole, respectively.[39,40]

DETECTION SYSTEMS

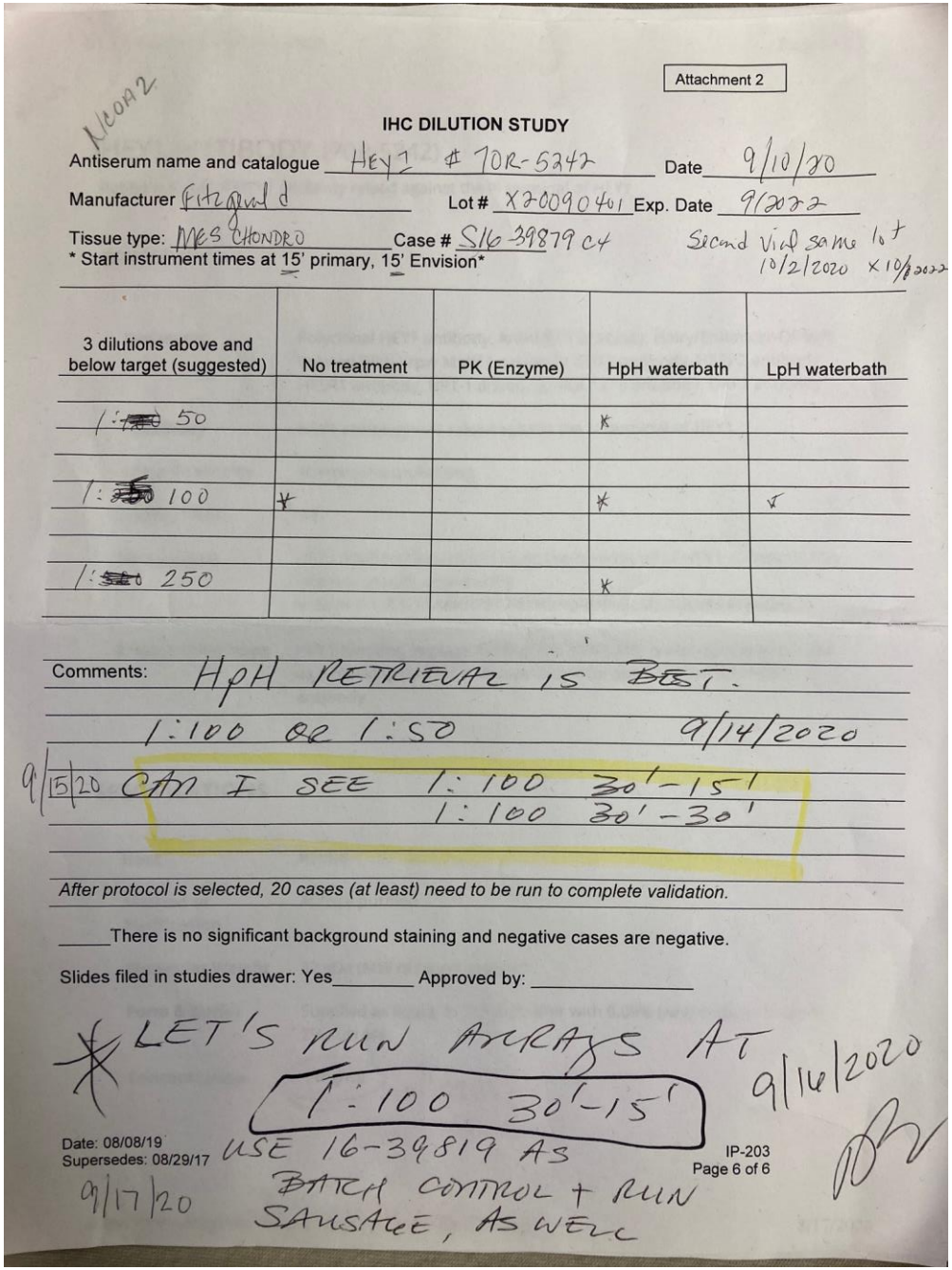
Numerous methods are available for detecting bound primary antibodies. The most commonly used methods are indirect enzyme labeling using peroxidase or alkaline phosphatase. Examples include peroxidase-antiperoxidase (PAP), alkaline phosphatase-antialkaline phosphatase (APAAP), labeled streptavidin-biotin peroxidase (LSAB), and avidin-biotin complex (ABC) methods. Peroxidase methods seem to be favored for use in most commercial automated immunostainers in clinical laboratories. New amplification techniques are also becoming available, including dextran polymer conjugates and the tyramide system; these techniques can potentially increase sensitivity by factors of 100 or more.[31,41,42] These newer, catalyzed, signal amplification techniques and catalyzed, reporter deposition techniques have the potential to offer extreme sensitivity, which most users are not accustomed to. A novel amplification system that makes use of mutually attractive antibodies based on species specificities has also recently been described.[43] This system reportedly increases sensitivity up to 200-fold and avoids use of avidin-biotin detection. A drawback is the additional time required for the multiple antibody incubation steps. Whether these newer methods will be practical and affordable for routine use in the automated clinical laboratory remains to be seen.

Chromogen selection can also be important. With the alkaline phosphatase system, one may use 5-bromo-4-chloro-3-indonyl phosphatase/nitroblue tetrazolium (BCIP/NBT) to produce a blue-purple product or fast red/naphthol AS-TR phosphate to give a red product. Peroxidase systems generally use 3,3′-diaminobenzidine (DAB) (brown) or 3-amino-9-ethylcarbazole (AEC) (red) chromogens. The choice of chromogen may be influenced by personal preference (red vs blue vs brown), sensitivity issues (peroxidase/DAB tends to be more sensitive than phosphatase/fast red), safety issues (DAB is a carcinogen), or technical issues (AEC is soluble in organic solvents and cannot be used with xylene-based mounting medium). We have found DAB to be a reliable chromogen that produces crisp, well-localized reactions that are permanent. Because DAB is a carcinogen, technicians must handle this chromogen carefully to eliminate the possibility of skin or mucous membrane contact.

FORMAL VALIDATION

Once one has decided to bring a new antibody into the clinical laboratory and has worked out the optimal staining conditions, the antibody should be validated on a set of clinical cases designed to test the diagnostic utility (sensitivity and specificity) of the antibody. In many cases, the literature provides a guide as to this utility. Although NCCLS guidelines require that antibodies undergo a validation process in individual laboratories, the details of this validation are left to the laboratory director.[6] Thus, one is left balancing the ideal situation of running a com-

UIHC Optimization



Attachment 2

IHC DILUTION STUDY

Antiserum name and catalogue HEY1 # 70R-5242 Date 9/10/20

Manufacturer Fitzgerald Lot # X20090401 Exp. Date 9/2022

Tissue type: MES CHONDRO Case # S16-39879 C4

* Start instrument times at 15' primary, 15' Envision*

Second vial same lot 10/2/2020 x 10/2022

3 dilutions above and below target (suggested)	No treatment	PK (Enzyme)	HpH waterbath	LpH waterbath
1:~~100~~ 50			*	
1:~~250~~ 100	*		*	*
1:~~500~~ 250			*	

Comments: HpH RETRIEVAL IS BEST.

1:100 OR 1:50 9/14/2020

9/15/20 CAN I SEE 1:100 30'-15'
1:100 30'-30'

After protocol is selected, 20 cases (at least) need to be run to complete validation.

_____There is no significant background staining and negative cases are negative.

Slides filed in studies drawer: Yes_____ Approved by: _____

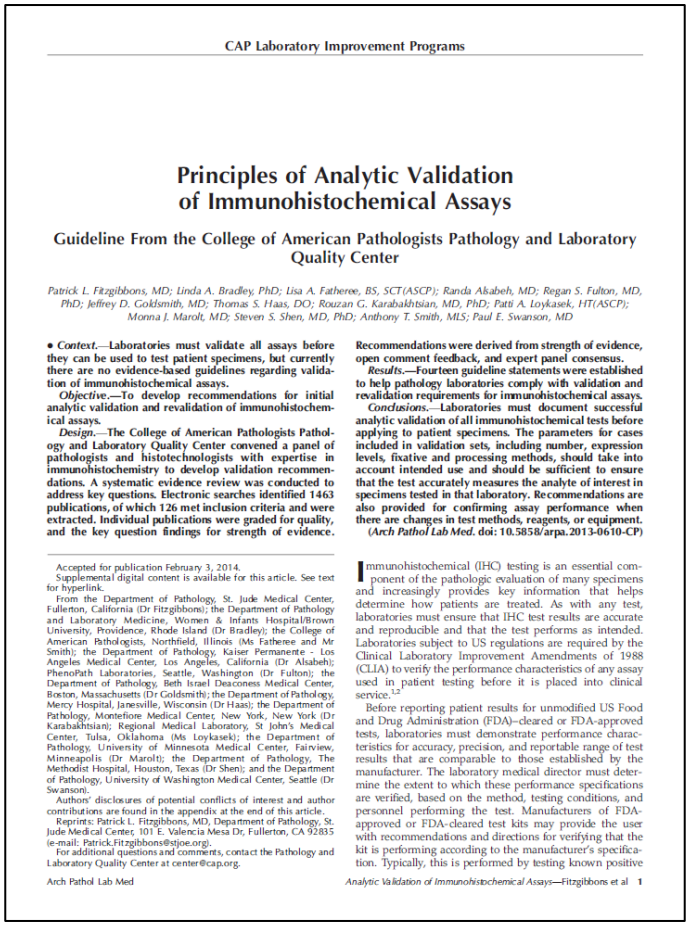
* LET'S RUN ARRAYS AT 1:100 30'-15' 9/16/2020

Date: 08/08/19
Supersedes: 08/29/17

USE 16-39819 AS BATCH CONTROL + RUN SAUSAGE, AS WELL 9/17/20

IP-203
Page 6 of 6

Validation



CAP Laboratory Improvement Programs

Principles of Analytic Validation of Immunohistochemical Assays

Guideline From the College of American Pathologists Pathology and Laboratory Quality Center

Patrick L. Fitzgibbons, MD; Linda A. Bradley, PhD; Lisa A. Fatheree, BS, SCT(ASCP); Randa Alsabeh, MD; Regan S. Fulton, MD, PhD; Jeffrey D. Goldsmith, MD; Thomas S. Haas, DO; Rouzan G. Karabakhtsian, MD, PhD; Patti A. Loykasek, HT(ASCP); Monna J. Marolt, MD; Steven S. Shen, MD, PhD; Anthony T. Smith, MLS; Paul E. Swanson, MD

• *Context.*—Laboratories must validate all assays before they can be used to test patient specimens, but currently there are no evidence-based guidelines regarding validation of immunohistochemical assays.

Objective.—To develop recommendations for initial analytic validation and revalidation of immunohistochemical assays.

Design.—The College of American Pathologists Pathology and Laboratory Quality Center convened a panel of pathologists and histotechnologists with expertise in immunohistochemistry to develop validation recommendations. A systematic evidence review was conducted to address key questions. Electronic searches identified 1463 publications, of which 126 met inclusion criteria and were extracted. Individual publications were graded for quality, and the key question findings for strength of evidence. Recommendations were derived from strength of evidence, open comment feedback, and expert panel consensus.

Results.—Fourteen guideline statements were established to help pathology laboratories comply with validation and revalidation requirements for immunohistochemical assays.

Conclusions.—Laboratories must document successful analytic validation of all immunohistochemical tests before applying to patient specimens. The parameters for cases included in validation sets, including number, expression levels, fixative and processing methods, should take into account intended use and should be sufficient to ensure that the test accurately measures the analyte of interest in specimens tested in that laboratory. Recommendations are also provided for confirming assay performance when there are changes in test methods, reagents, or equipment.

(*Arch Pathol Lab Med.* doi: 10.5858/arpa.2013-0610-CP)

Accepted for publication February 3, 2014.

Supplemental digital content is available for this article. See text for hyperlink.

From the Department of Pathology, St. Jude Medical Center, Fullerton, California (Dr Fitzgibbons); the Department of Pathology and Laboratory Medicine, Women & Infants Hospital/Brown University, Providence, Rhode Island (Dr Bradley); the College of American Pathologists, Northfield, Illinois (Ms Fatheree and Mr Smith); the Department of Pathology, Kaiser Permanente - Los Angeles Medical Center, Los Angeles, California (Dr Alsabeh); PhenoPath Laboratories, Seattle, Washington (Dr Fulton); the Department of Pathology, Beth Israel Deaconess Medical Center, Boston, Massachusetts (Dr Goldsmith); the Department of Pathology, Mercy Hospital, Janesville, Wisconsin (Dr Haas); the Department of Pathology, Montefiore Medical Center, New York, New York (Dr Karabakhtsian); Regional Medical Laboratory, St John's Medical Center, Tulsa, Oklahoma (Ms Loykasek); the Department of Pathology, University of Minnesota Medical Center, Fairview, Minneapolis (Dr Marolt); the Department of Pathology, The Methodist Hospital, Houston, Texas (Dr Shen); and the Department of Pathology, University of Washington Medical Center, Seattle (Dr Swanson).

Authors' disclosures of potential conflicts of interest and author contributions are found in the appendix at the end of this article.

Reprints: Patrick L. Fitzgibbons, MD, Department of Pathology, St. Jude Medical Center, 101 E. Valencia Mesa Dr, Fullerton, CA 92835 (e-mail: Patrick.Fitzgibbons@stjoe.org).

For additional questions and comments, contact the Pathology and Laboratory Quality Center at center@cap.org.

Immunohistochemical (IHC) testing is an essential component of the pathologic evaluation of many specimens and increasingly provides key information that helps determine how patients are treated. As with any test, laboratories must ensure that IHC test results are accurate and reproducible and that the test performs as intended. Laboratories subject to US regulations are required by the Clinical Laboratory Improvement Amendments of 1988 (CLIA) to verify the performance characteristics of any assay used in patient testing before it is placed into clinical service.[1,2]

Before reporting patient results for unmodified US Food and Drug Administration (FDA)–cleared or FDA-approved tests, laboratories must demonstrate performance characteristics for accuracy, precision, and reportable range of test results that are comparable to those established by the manufacturer. The laboratory medical director must determine the extent to which these performance specifications are verified, based on the method, testing conditions, and personnel performing the test. Manufacturers of FDA-approved or FDA-cleared test kits may provide the user with recommendations and directions for verifying that the kit is performing according to the manufacturer's specification. Typically, this is performed by testing known positive

Category	Validation Cohort
Diagnostic*	10+/10-
Predictive*	20+/20-
ER/HER2 FDA**	20+/20-
ER/HER2 LDT**	40+/40-

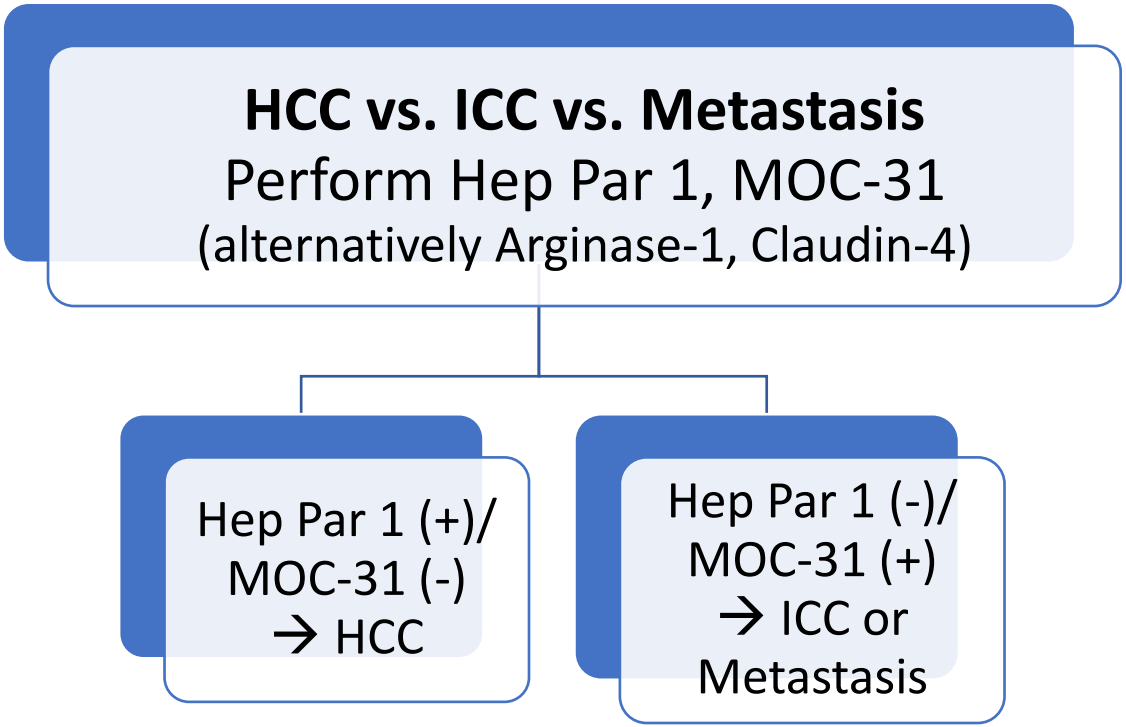
* ≥90% concordance with expected results

** ≥90% concordance with expected positives and ≥95% concordance with expected negatives

Everything you cram and then immediately forget after you take Step I . . . I make that into a diagnostic test.

--@IHC_guy to his pathology small group a few years ago

Dx Algorithm: Poorly Differentiated Carcinoma in the Liver



IHC menu for this application:

HCC Markers:
- Hep Par 1
- GPC3
- pCEA/CD10 (canalicular)
- Arginase-1

Non-HCC Markers:
- MOC-31
- Claudin-4

Hep Par 1 was Raised Against FFPE Tissue from a Failed Liver Transplant and 15-Years Later was Shown to Recognize CPS1

American Journal of Pathology, Vol. 143, No. 4, October 1993

Technical Advances

Hepatocyte Paraffin 1: A Monoclonal Antibody that Reacts with Hepatocytes and Can Be Used for Differential Diagnosis of Hepatic Tumors

Anne E. Wennerberg,* Michael A. Nalesnik,* and William B. Coleman†

From the Division of Transplant Pathology, Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, and the Department of Pathology,† University of North Carolina at Chapel Hill, Chapel Hill, North Carolina*

Hepatocyte paraffin 1 is a monoclonal antibody that has been developed specifically to react with hepatocytes in routine formalin-fixed and paraffin-embedded surgical pathology tissues. It results in a distinct, granular cytoplasmic staining of hepatocytes but fails to react with bile ducts and nonparenchymal liver cells. The antibody decorates a majority of hepatocellular carcinomas, including fibrolamellar variants. It fails to react with a wide variety of other adult malignancies, with the exception of focal staining in a few gastrointestinal malignancies, including a subpopulation of gastric carcinomas. (Am J Pathol 1993, 143:1050–1054)

Panels of antibodies useful in evaluating primary and secondary liver tumors include α-fetoprotein, α-1-antitrypsin, monoclonal carcinoembryonic antigen, polyclonal carcinoembryonic antigen cross-reactive for biliary glycoprotein, antibodies to cytokeratins, and neuroendocrine markers such as chromogranin.[1–3] We have developed a new monoclonal antibody, hepatocyte paraffin 1 (Hep Par 1), that reacts with both normal and neoplastic hepatocytes in routine formalin-fixed, paraffin-embedded tissues. We have evaluated this antibody in a retrospective analysis of adult human tumors, with and without metastases, and find that it is conserved in a majority of hepatocellular carcinomas (HCCs).

Materials and Methods

Immunizations and Fusion

The immunogen was obtained from a 10% neutral buffered, formalin-fixed, failed allograft liver that was mechanically disrupted. Six-week-old female Balb/C mice received serial injections of the immunogen. Animal use protocols had prior approval of the Institutional Animal Care and Use Committee of the University of Pittsburgh. Fusion was by a standard method and based on a modification of Kohler and Milstein.[4] The myeloma cells (P3X63-Ag8.653) and fusion protocols were a generous gift from Dr. Howard Reisner (UNC Chapel Hill, NC).[5–7]

Screening

Positive wells were screened by evaluating staining patterns of supernatants on microscope slides, containing multiple sections of nondigested, formalin-fixed human tissues, by immunoperoxidase using a mouse immunoglobulin G (IgG) ABC kit (Vector Laboratories, Burlingame, CA). One well that was highly specific for adult and fetal liver was subjected to single cell cloning and is the subject of this report.

Supported by the Pathology Education and Research Foundation of the University of Pittsburgh School of Medicine. WBC was supported by NIH training grant T32 ES 07017.

Accepted for publication June 22, 1993.

Address reprint requests to: Dr. Anne E. Wennerberg, Division of Transplant Pathology, Department of Pathology, University of Pittsburgh School of Medicine, 720 Scaife Hall, Pittsburgh, PA 15261.

Laboratory Investigation (2008) 88, 78–88

The antigen for Hep Par 1 antibody is the urea cycle enzyme carbamoyl phosphate synthetase 1

Samantha L Butler*, Huijia Dong*, Diana Cardona, Minghong Jia, Ran Zheng, Haizhen Zhu, James M Crawford and Chen Liu

Hepatocyte paraffin 1 (Hep Par 1), a murine monoclonal antibody, is widely used in surgical pathology practice to determine the hepatocellular origin of neoplasms. However, identity of the antigen for Hep Par 1 is unknown. The aim of this study was to characterize the Hep Par 1 antigen. To identify the antigen, immunoprecipitation was used to isolate the protein from human liver tissue, and a distinct protein band was detected at approximately 165 kDa. The protein band was also present in small intestinal tissue, but was not present in several other non-liver tissues nor in three human hepatocellular carcinoma cell lines, Huh-7, HepG2, and LH86. The protein was purified and analyzed by mass spectrometry. It was identified as carbamoyl phosphate synthetase 1 (CPS1). CPS1 is a rate-limiting enzyme in urea cycle and is located in mitochondria. We demonstrated that hepatoid tumors (gastric and yolk sac) were immunoreactive with both Hep Par 1 antibody and anti-CPS1 antibody, further confirming the results of mass spectrometric analysis. We found that the three human hepatocellular carcinoma cell lines do not express either CPS1 RNA or protein. We confirmed that the gene was present in these cell lines, suggesting that suppression of CPS1 expression occurs at the transcriptional level. This finding may have relevance to liver carcinogenesis, since poorly differentiated hepatocellular carcinomas exhibit poor to absent immunoreactivity to Hep Par 1. In conclusion, we have identified the antigen for Hep Par 1 antibody as a urea cycle enzyme CPS1. Our results should encourage further investigation of potential role that CPS1 expression plays in liver pathobiology and carcinogenesis.

Laboratory Investigation (2008) **88**, 78–88; doi:10.1038/labinvest.3700699; published online 19 November 2007

KEYWORDS: Hep Par 1 antibody; CPS1; liver; urea cycle; liver cancer

The histological distinction between hepatocellular carcinomas (HCC) and metastatic adenocarcinoma to the liver can sometimes be a challenging dilemma for surgical pathologists, particularly given the histological variants of HCC that can occur. In addition, tumors in other sites can display hepatoid morphologic features, adding to the diagnostic challenge when considering their metastasis to the liver. In the end, a wide panel of immunohistochemical markers is often used for the differential diagnosis of HCC, cholangiocarcinoma and metastatic adenocarcinoma. These markers include alpha-fetoprotein (AFP), polyclonal carcinoembryonic antigen (pCEA), and alpha-1-antitrypsin.[1] None of these markers, however, are highly specific or sensitive for hepatocyte differentiation.

In 1993, Wennerberg *et al*,[2] developed a new monoclonal antibody specific for hepatocytes in formalin-fixed, paraffin-embedded tissues. This antibody, named hepatocyte paraffin 1 (Hep Par 1, clone OCH1E5.2.10), was generated using tissue extracts from a formalin-fixed failed allograft liver. Subsequent studies showed a high sensitivity and specificity of Hep Par 1 for normal hepatocytes and neoplastic hepatic tissue.[3–6] Additional recent studies examined Hep Par 1 reactivity in a variety of non-hepatic tissues, both benign and neoplastic.[7–10] A large number of gastric adenocarcinomas show Hep Par 1 reactivity.[11] Rare examples of cholangiocarcinomas, yolk sac tumor, and adenocarcinomas of the ovary, adrenal cortex, lung, endocervix, colon, and pancreas have shown focal Hep Par 1 staining.[8,12,13] Hep Par 1 reactivity is also seen in benign small intestinal mucosa and intestinal metaplasia of the esophagus and stomach.[14]

Although the Hep Par 1 antibody exhibits excellent sensitivity and specificity for well-differentiated hepatocytes,

Department of Pathology, Immunology and Laboratory Medicine, University of Florida, Gainesville, FL, USA
Correspondence: Dr C Liu, MD, PhD, Department of Pathology, Immunology and Laboratory Medicine, University of Florida, 1600 SW Archer Rd, PO Box 100275, Gainesville, Florida 32610, USA. E-mail: Liu@pathology.ufl.edu
*These authors contributed equally to this work.

Received 07 August 2007; revised 15 October 2007; accepted 15 October 2007

Am J Surg Pathol. 2010 Aug;34(8):1147-54. doi: 10.1097/PAS.0b013e3181e5dffa.

Arginase-1: a new immunohistochemical marker of hepatocytes and hepatocellular neoplasms.

Yan BC[1], Gong C, Song J, Krausz T, Tretiakova M, Hyjek E, Al-Ahmadie H, Alves V, Xiao SY, Anders RA, Hart JA.

Author information

Abstract

The distinction of hepatocellular carcinoma (HCC) from metastatic tumor in the liver often presents a diagnostic challenge that carries significant impact on prognostication and therapy. The number of diagnostically useful immunohistochemical markers of hepatocytes is limited to hepatocyte paraffin antigen (HepPar-1), polyclonal carcinoembryonic antigen, and CD10, with alpha-fetoprotein and glypican-3 labeling HCCs. Arginase-1 (Arg-1) is a binuclear manganese metalloenzyme that catalyzes the hydrolysis of arginine to ornithine and urea. We used immunohistochemistry to compare the sensitivity of Arg-1 to that of HepPar-1 in 151 HCCs. We found that the overall sensitivities of Arg-1 and HepPar-1 are 96.0% and 84.1%, respectively. The sensitivities of Arg-1 in well, moderately, and poorly differentiated HCCs are 100%, 96.2%, and 85.7%, respectively, whereas, in comparison, HepPar-1 demonstrated sensitivities of 100%, 83.0%, and 46.4% for well, moderately, and poorly differentiated tumors, respectively. There were no HCCs in our study that were reactive for HepPar-1 but nonreactive for Arg-1. We also examined Arg-1 expression in nonhepatocellular tumors, including many that are potential mimics of HCC (renal cell carcinomas, neuroendocrine tumors, melanomas, gastric adenocarcinomas, and adrenocortical carcinomas) and found that only 2 non-HCC tumors were reactive for Arg-1. Arg-1 represents a sensitive and specific marker of benign and malignant hepatocytes that may ultimately prove to be a useful diagnostic tool in routine surgical pathology practice.

PMID: 20661013 PMCID: PMC3160135 DOI: 10.1097/PAS.0b013e3181e5dffa

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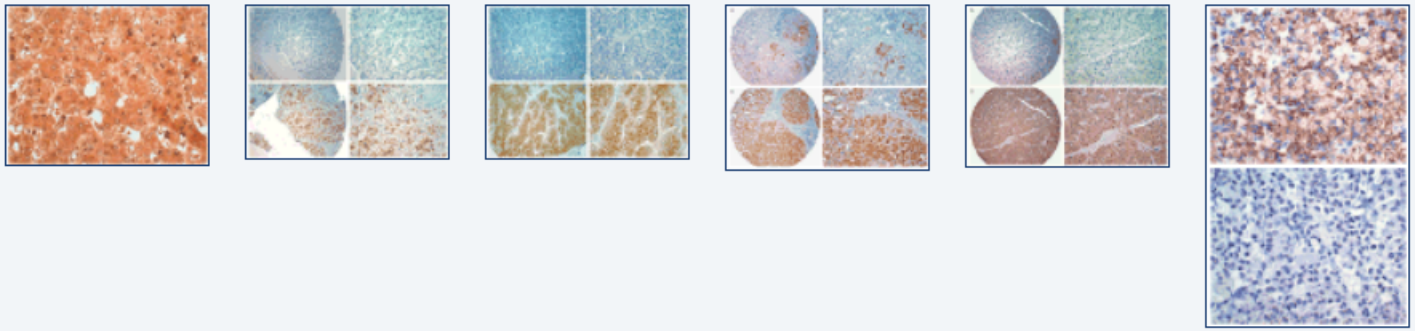
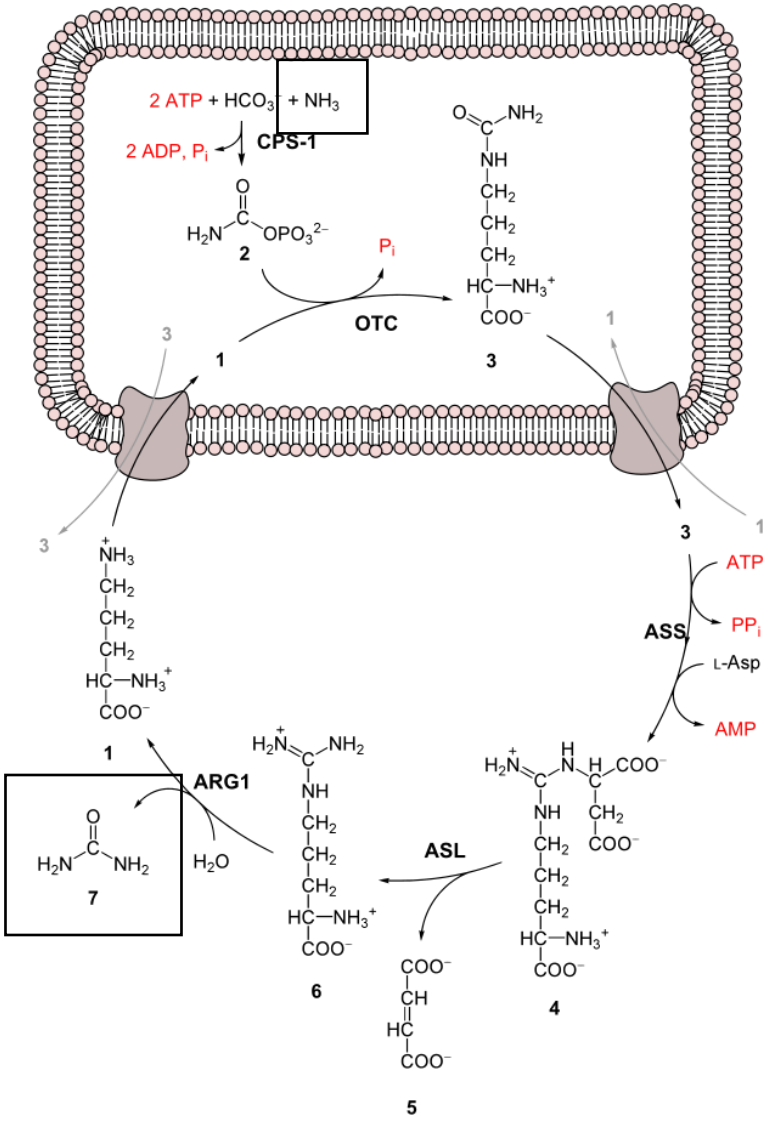


Urea Cycle



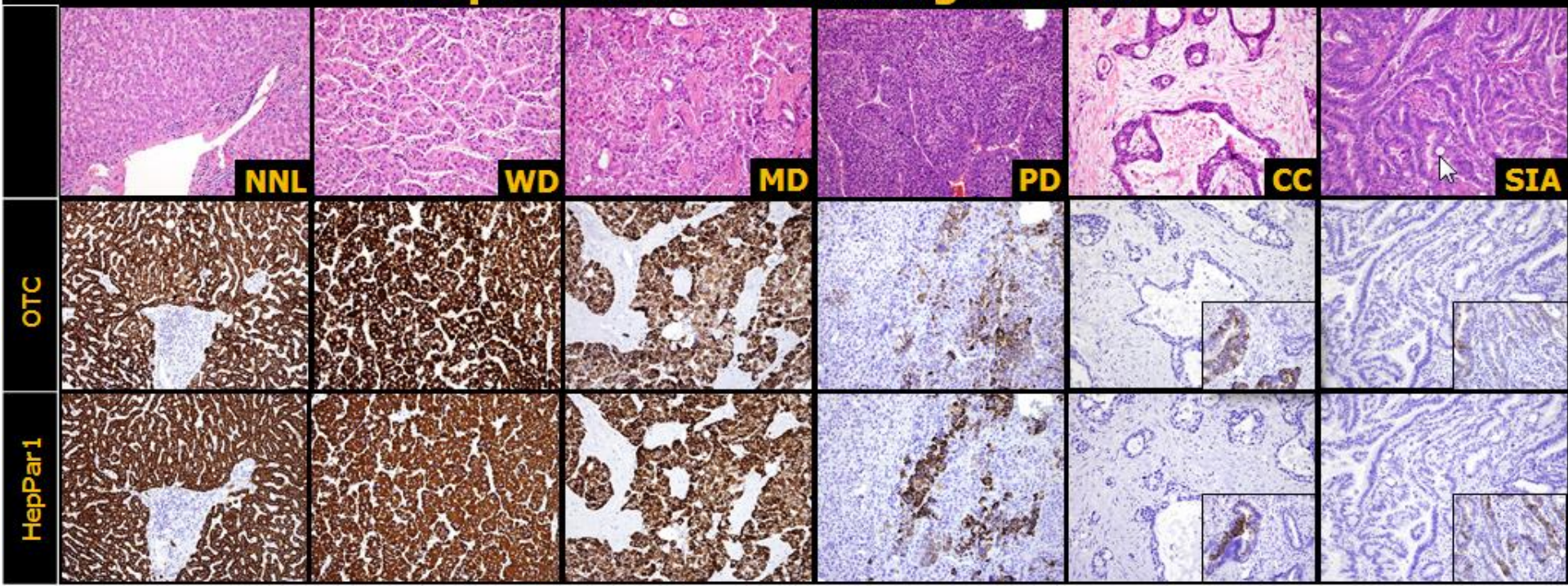
Abbrev.	Enzyme
CPS1	carbamoyl phosphate synthetase I
OTC	ornithine transcarbamoylase
ASS	argininosuccinate synthetase
ASL	argininosuccinate lyase
ARG1	arginase 1

Number	Molecule
1	L-ornithine
2	carbamoyl phosphate
3	L-citrulline
4	argininosuccinate
5	fumarate
6	L-arginine
7	urea

Detailed Expression Data: Percent Positive (Mean H-score, if positive)

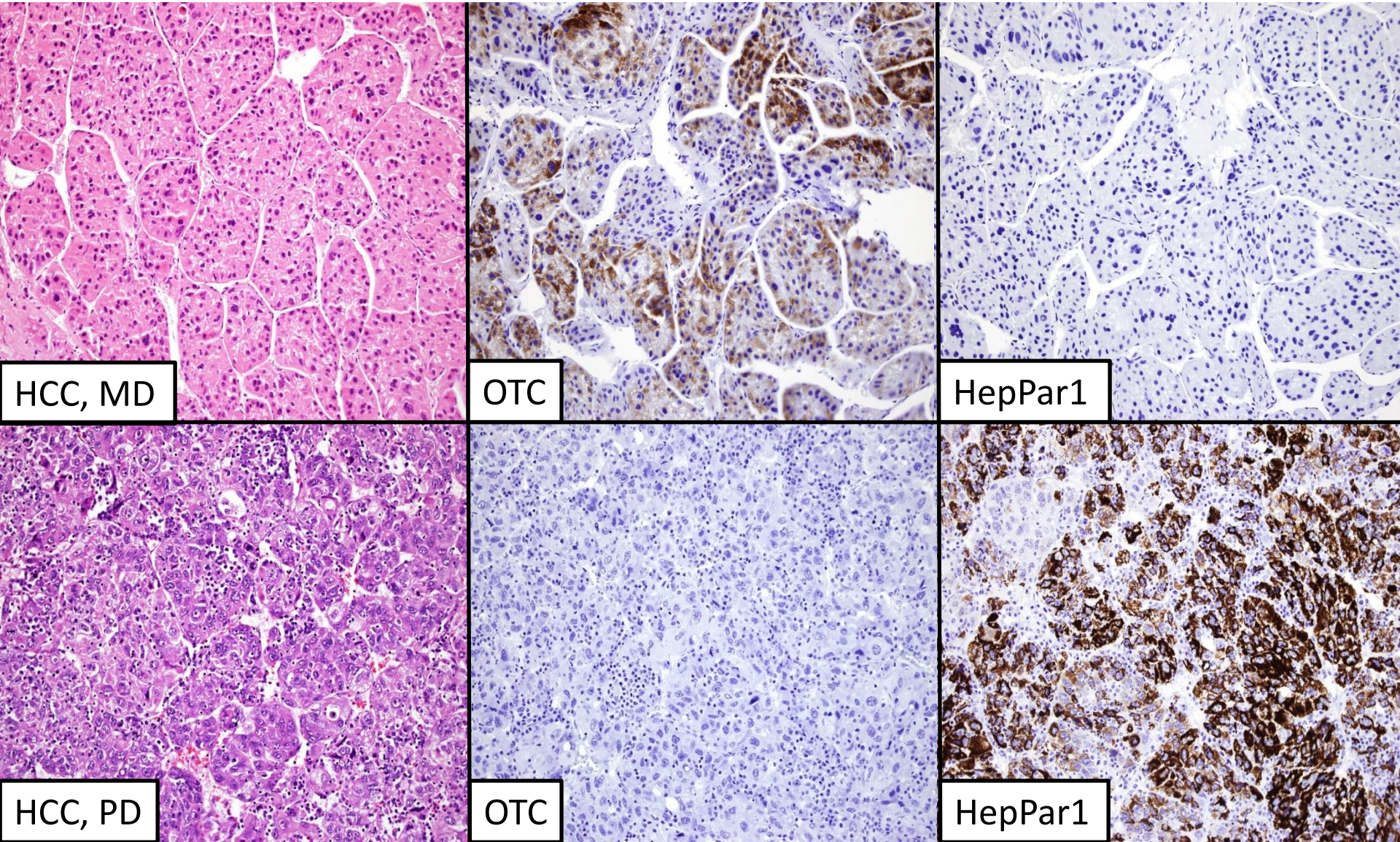
	Diagnosis	OTC	HepPar1	ARG	GPC3	ASL
	NNL (n=99)	100% (291)	99% (298)	100% (280)	1% (13)	100% (296)
Benign liver lesions	DN (n=8)	100% (278)	100% (291)	100% (239)	13% (3)	100% (291)
	FNH (n=32)	100% (290)	Not done	97% (281)	Not done	100% (299)
	HA (n=45)	100% (265)	Not done	100% (253)	Not done	100% (297)
HCC	WD (n=34)	97% (248)	97% (270)	94% (195)	50% (67)	100% (292)
	MD (n=104)	94% (248)	92% (235)	79% (149)	60% (122)	99% (278)
	PD (n=24)	75% (197)	79% (204)	75% (145)	71% (177)	100% (263)
Other tumors	CC (n=135)	20% (75)	20% (94)	1.5% (67)	8% (49)	95% (250)
	SIA (n=93)	38% (125)	42% (127)	0% (0)	2% (9)	98% (260)

Representative Staining Patterns



There were 5 OTC+/HepPar1- and 4 OTC-/HepPar1+ HCCs

Adding OTC to HepPar1 Increased Sensitivity from 91% to 94%



Pathology International

Pathology International 2016; **66**: 333–336 doi:10.1111/pin.12414

Original Article

Loss of Hep Par 1 immunoreactivity in the livers of patients with carbamoyl phosphate synthetase 1 deficiency

Maki Yamaguchi,[1] Tatsuki R. Kataoka,[2] Takahiro Shibayama,[1] Akinari Fukuda,[3] Atsuko Nakazawa,[4] Sachiko Minamiguchi,[1] Takaki Sakurai,[1] Aya Miyagawa-Hayashino,[1] Toru Yorifuji,[5] Mureo Kasahara,[3] Shinji Uemoto[2] and Hironori Haga[1]

[1]*Department of Diagnostic Pathology,* [2]*Organ Transplant Unit, Kyoto University Hospital, Kyoto,* [3]*Organ Transplantation Center,* [4]*Division of Clinical Pathology, National Center for Child Health and Development, Tokyo, and* [5]*Department of Pediatrics, Osaka City General Hospital, Osaka, Japan*

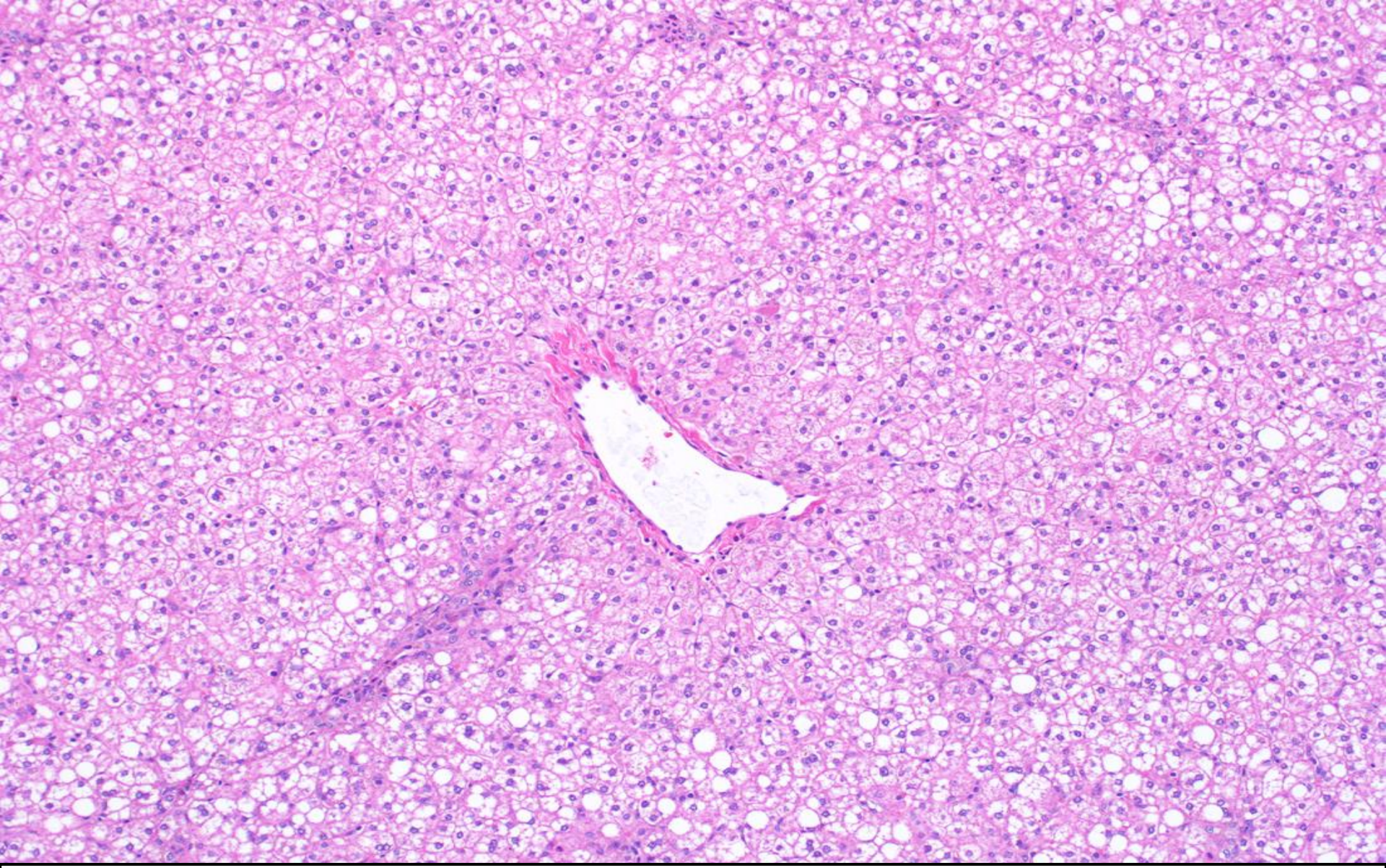
The hepatocyte paraffin 1 (Hep Par 1) antibody is widely used as a hepatocyte marker, recognizing carbamoyl phosphate synthetase 1 (CPS1), an essential component of the urea cycle. Various missense, nonsense, and frameshift mutations occur in the *CPS1* gene. In neonatal patients with homozygous CPS1 deficiency (CPS1D), urea cycle defects with resulting severe hyperammonemia can be fatal, though liver transplantation provides a complete cure for CPS1D. We performed Hep Par 1 immunostaining in the explanted livers of 10 liver transplant patients with CPS1D. Seven were negative for Hep Par 1 in the hepatocytes and the other three showed normal diffuse granular cytoplasmic staining. As expected, all three Hep Par 1-positive patients had at least one missense mutation, and all four patients who had only nonsense or frameshift mutations were Hep Par 1-negative. The other three patients were unexpectedly negative for Hep Par 1, even though each had one missense mutation. These results suggest that CPS1D can be related to the loss of Hep Par 1 reactivity due to the loss of protein production, a one amino acid substitution resulting in an abortive protein product, or both. Hep Par 1 immunohistochemistry can be used as a simple method to confirm CPS1D.

Key words: *CPS1*, Hep Par 1, liver transplantation

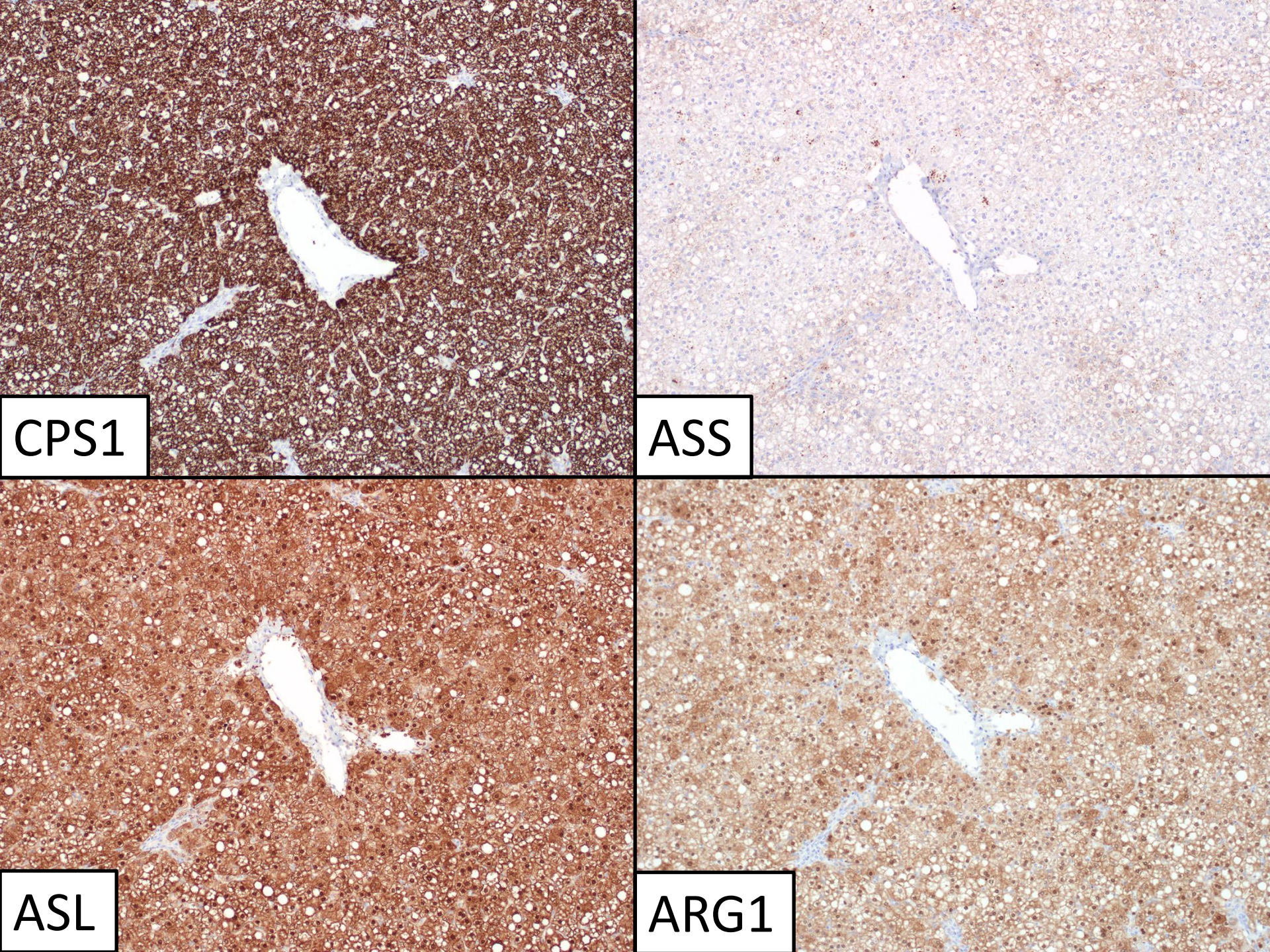
Correspondence: Tatsuki R. Kataoka, MD & PhD, Organ Transplantation Unit, Kyoto University Hospital, Sakyo-ku, Kyoto, 606-8507, Japan. E-mail: trkata@kuhp.kyoto-u.ac.jp

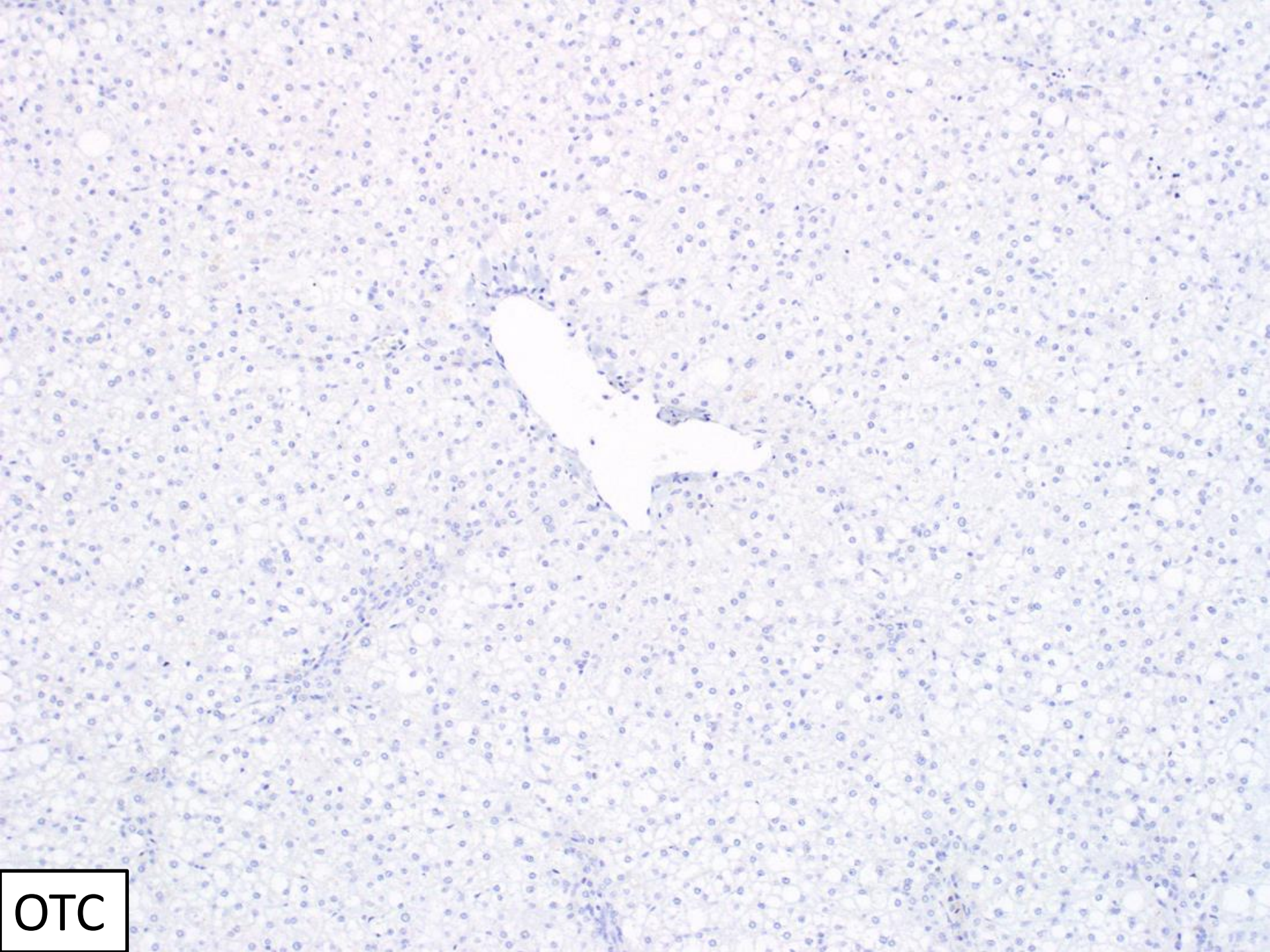
Received 4 February 2016. Accepted for publication 5 April 2016.

Hepatocyte paraffin 1 (Hep Par 1, clone OCH1E5.2.10) is a mouse monoclonal antibody generated using extracts from formalin-fixed liver tissue.[1] The antibody reacts with the mitochondria of hepatocytes and small intestine epithelium, but not with that of most other tissues.[1,2] Therefore, this antibody has been used as a marker for hepatocytes and hepatocellular neoplasms in surgical pathology practice. More than a decade after the development of the monoclonal antibody, the antigen was identified as carbamoyl phosphate synthetase 1 (CPS1).[3] CPS1 is the rate-limiting enzyme in the urea cycle, synthesizing carbamoyl phosphate from bicarbonate, adenosine triphosphate and ammonia.[4] This enzyme is the most abundant protein in liver mitochondria, accounting for about 20 % of the total mitochondrial proteins.[4] This enzyme is composed of an intersubunit interacting domain, a glutaminase domain, a bicarbonate phosphorylation domain, a domain of unknown function, a carbamate phosphorylation domain and an N-acetyl-L-glutamate binding domain,[5] which the Hep Par 1 antibody recognizes is not known. CPS1 deficiency (CPS1D; OMIM #237300) is an autosomal recessive disorder characterized by hyperammonemia ranging from neonatally lethal to environmentally-induced adult-onset disease.[4,5] In newborn patients, hyperammonemia manifests after feeding commencement, with symptoms such as vomiting, hypothermia, somnolence, lethargy, apnea, seizure, or coma. Though the initial medical treatment of CPS1D consists of protein restriction and medications such as sodium phenylbutyrate, only liver transplantation can offer complete avoidance of recurrent hyperammonemia and prevent serious neurological damage.[6] Various types of mutations causing CPS1D have been reported, but missense mutations are the most frequently observed.[5] Other mutations include small deletions, splice site changes, nonsense mutations, and small insertions.[5] To our knowledge, Hep Par 1 immunoreactivity has not been studied in the livers of patients with CPS1D; therefore, the aim of our study was to



4-day-old boy presented comatose with hyperammonemia (962 µg/dl – RR 15-45 µg/dl); transplanted; biochemical dx of OTC deficiency; missense alteration in exon 9; alive at 15-years follow up

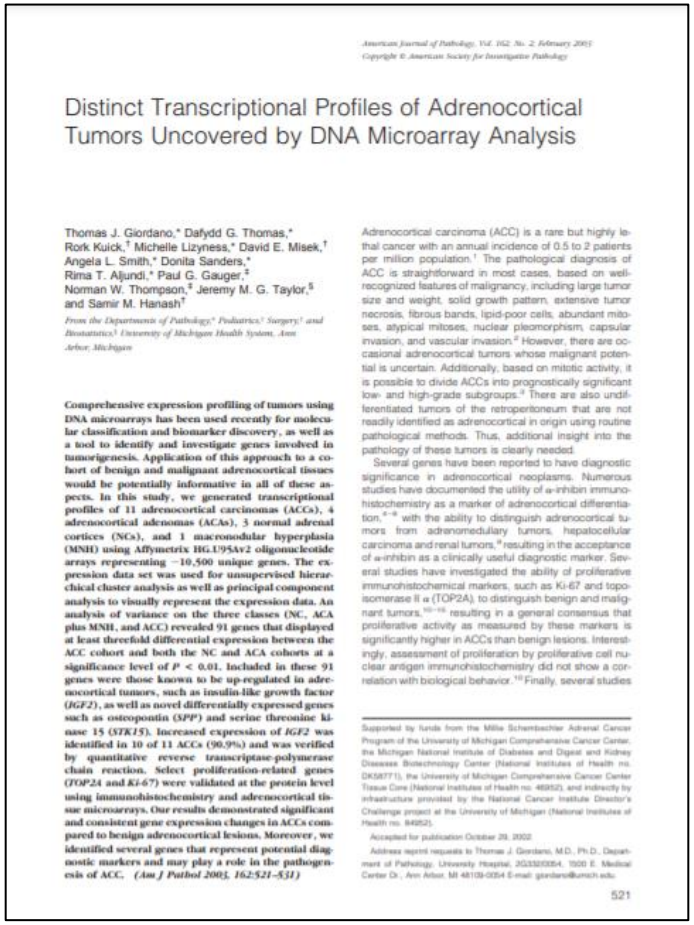

CPS1
ASS
ASL
ARG1


OTC

Hey, what do you know about IGF2 immunohistochemistry?

--Jason L Hornick, MD, PhD

IGF2 overexpression is associated with adrenal cortical carcinoma and Beckwith-Wiedemann Syndrome



American Journal of Pathology, Vol. 162, No. 2, February 2003

Distinct Transcriptional Profiles of Adrenocortical Tumors Uncovered by DNA Microarray Analysis

Thomas J. Giordano,* Dafydd G. Thomas,* Rork Kuick,† Michelle Lizyness,* David E. Misek,† Angela L. Smith,* Donita Sanders,* Rima T. Aljundi,* Paul G. Gauger,‡ Norman W. Thompson,‡ Jeremy M. G. Taylor,§ and Samir M. Hanash†

From the Departments of Pathology, Pediatrics,† Surgery,‡ and Biostatistics,§ University of Michigan Health System, Ann Arbor, Michigan*

Comprehensive expression profiling of tumors using DNA microarrays has been used recently for molecular classification and biomarker discovery, as well as a tool to identify and investigate genes involved in tumorigenesis. Application of this approach to a cohort of benign and malignant adrenocortical tissues would be potentially informative in all of these aspects. In this study, we generated transcriptional profiles of 11 adrenocortical carcinomas (ACCs), 4 adrenocortical adenomas (ACAs), 3 normal adrenal cortices (NCs), and 1 macronodular hyperplasia (MNH) using Affymetrix HG-U95Av2 oligonucleotide arrays representing ~10,500 unique genes. The expression data set was used for unsupervised hierarchical cluster analysis as well as principal component analysis to visually represent the expression data. An analysis of variance on the three classes (NC, ACA plus MNH, and ACC) revealed 91 genes that displayed at least threefold differential expression between the ACC cohort and both the NC and ACA cohorts at a significance level of $P < 0.01$. Included in these 91 genes were those known to be up-regulated in adrenocortical tumors, such as insulin-like growth factor (*IGF2*), as well as novel differentially expressed genes such as osteopontin (*SPP1*) and serine threonine kinase 15 (*STK15*). Increased expression of *IGF2* was identified in 10 of 11 ACCs (90.9%) and was verified by quantitative reverse transcriptase-polymerase chain reaction. Select proliferation-related genes (*TOP2A* and *Ki-67*) were validated at the protein level using immunohistochemistry and adrenocortical tissue microarrays. Our results demonstrated significant and consistent gene expression changes in ACCs compared to benign adrenocortical lesions. Moreover, we identified several genes that represent potential diagnostic markers and may play a role in the pathogenesis of ACC. (*Am J Pathol 2003, 162:521–531*)

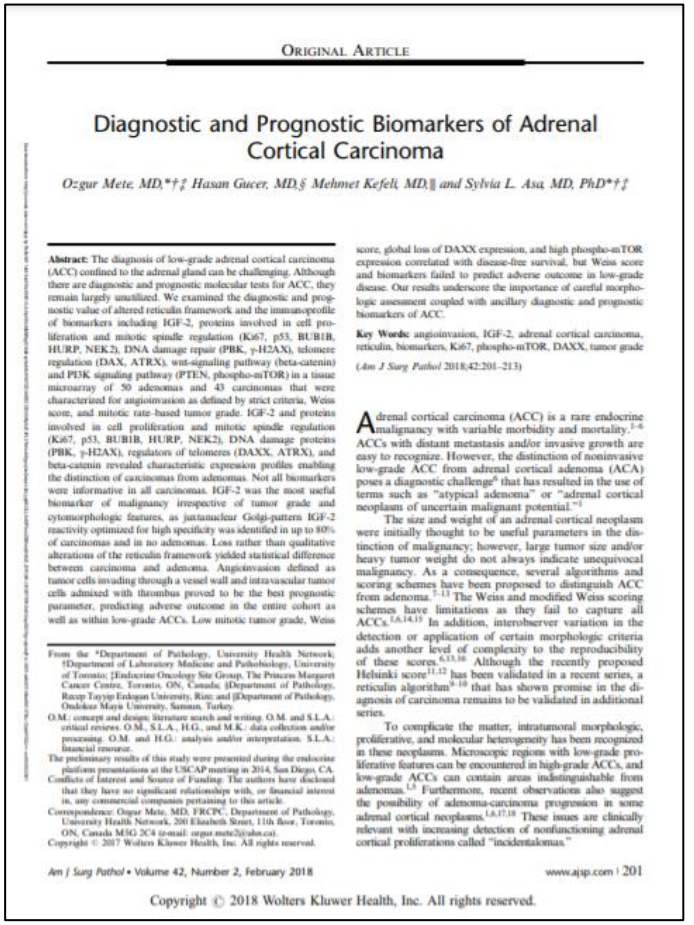
Adrenocortical carcinoma (ACC) is a rare but highly lethal cancer with an annual incidence of 0.5 to 2 patients per million population.[1] The pathological diagnosis of ACC is straightforward in most cases, based on well-recognized features of malignancy, including large tumor size and weight, solid growth pattern, extensive tumor necrosis, fibrous bands, lipid-poor cells, abundant mitoses, atypical mitoses, nuclear pleomorphism, capsular invasion, and vascular invasion.[2] However, there are occasional adrenocortical tumors whose malignant potential is uncertain. Additionally, based on mitotic activity, it is possible to divide ACCs into prognostically significant low- and high-grade subgroups.[3] There are also undifferentiated tumors of the retroperitoneum that are not readily identified as adrenocortical in origin using routine pathological methods. Thus, additional insight into the pathology of these tumors is clearly needed.

Several genes have been reported to have diagnostic significance in adrenocortical neoplasms. Numerous studies have documented the utility of α-inhibin immunohistochemistry as a marker of adrenocortical differentiation,[4–6] with the ability to distinguish adrenocortical tumors from adrenomedullary tumors, hepatocellular carcinoma and renal tumors,[6] resulting in the acceptance of α-inhibin as a clinically useful diagnostic marker. Several studies have investigated the ability of proliferative immunohistochemical markers, such as Ki-67 and topoisomerase II α (TOP2A), to distinguish benign and malignant tumors,[10–13] resulting in a general consensus that proliferative activity as measured by these markers is significantly higher in ACCs than benign lesions. Interestingly, assessment of proliferation by proliferative cell nuclear antigen immunohistochemistry did not show a correlation with biological behavior.[10] Finally, several studies

Supported by funds from the Millie Schembechler Adrenal Cancer Program of the University of Michigan Comprehensive Cancer Center, the Michigan National Institute of Diabetes and Digest and Kidney Diseases Biotechnology Center (National Institutes of Health no. DK58777), the University of Michigan Comprehensive Cancer Center Tissue Core (National Institutes of Health no. 46852), and indirectly by infrastructure provided by the National Cancer Institute Director's Challenge project at the University of Michigan (National Institutes of Health no. 84852).

Accepted for publication October 29, 2002.

Address reprint requests to Thomas J. Giordano, M.D., Ph.D., Department of Pathology, University Hospital, 2G332/0054, 1500 E. Medical Center Dr., Ann Arbor, MI 48109-0054. E-mail: giordano@umich.edu.



ORIGINAL ARTICLE

Diagnostic and Prognostic Biomarkers of Adrenal Cortical Carcinoma

Ozgur Mete, MD,†‡ Hasan Gucer, MD,§ Mehmet Kefeli, MD,‖ and Sylvia L. Asa, MD, PhD*†‡*

Abstract: The diagnosis of low-grade adrenal cortical carcinoma (ACC) confined to the adrenal gland can be challenging. Although there are diagnostic and prognostic molecular tests for ACC, they remain largely unutilized. We examined the diagnostic and prognostic value of altered reticulin framework and the immunoprofile of biomarkers including IGF-2, proteins involved in cell proliferation and mitotic spindle regulation (Ki67, p53, BUB1B, HURP, NEK2), DNA damage repair (PBK, γ-H2AX), telomere regulation (DAX, ATRX), wnt-signaling pathway (beta-catenin) and PI3K signaling pathway (PTEN, phospho-mTOR) in a tissue microarray of 50 adenomas and 43 carcinomas that were characterized for angioinvasion as defined by strict criteria, Weiss score, and mitotic rate–based tumor grade. IGF-2 and proteins involved in cell proliferation and mitotic spindle regulation (Ki67, p53, BUB1B, HURP, NEK2), DNA damage proteins (PBK, γ-H2AX), regulators of telomeres (DAXX, ATRX), and beta-catenin revealed characteristic expression profiles enabling the distinction of carcinomas from adenomas. Not all biomarkers were informative in all carcinomas. IGF-2 was the most useful biomarker of malignancy irrespective of tumor grade and cytomorphologic features, as juxtanuclear Golgi-pattern IGF-2 reactivity optimized for high specificity was identified in up to 80% of carcinomas and in no adenomas. Loss rather than qualitative alterations of the reticulin framework yielded statistical difference between carcinoma and adenoma. Angioinvasion defined as tumor cells invading through a vessel wall and intravascular tumor cells admixed with thrombus proved to be the best prognostic parameter, predicting adverse outcome in the entire cohort as well as within low-grade ACCs. Low mitotic tumor grade, Weiss score, global loss of DAXX expression, and high phospho-mTOR expression correlated with disease-free survival, but Weiss score and biomarkers failed to predict adverse outcome in low-grade disease. Our results underscore the importance of careful morphologic assessment coupled with ancillary diagnostic and prognostic biomarkers of ACC.

Key Words: angioinvasion, IGF-2, adrenal cortical carcinoma, reticulin, biomarkers, Ki67, phospho-mTOR, DAXX, tumor grade

(*Am J Surg Pathol* 2018;42:201–213)

Adrenal cortical carcinoma (ACC) is a rare endocrine malignancy with variable morbidity and mortality.[1–6] ACCs with distant metastasis and/or invasive growth are easy to recognize. However, the distinction of noninvasive low-grade ACC from adrenal cortical adenoma (ACA) poses a diagnostic challenge[6] that has resulted in the use of terms such as "atypical adenoma" or "adrenal cortical neoplasm of uncertain malignant potential."[4]

The size and weight of an adrenal cortical neoplasm were initially thought to be useful parameters in the distinction of malignancy; however, large tumor size and/or heavy tumor weight do not always indicate unequivocal malignancy. As a consequence, several algorithms and scoring schemes have been proposed to distinguish ACC from adenoma.[7–13] The Weiss and modified Weiss scoring schemes have limitations as they fail to capture all ACCs.[1,6,14,15] In addition, interobserver variation in the detection or application of certain morphologic criteria adds another level of complexity to the reproducibility of these scores.[6,13,16] Although the recently proposed Helsinki score[11,12] has been validated in a recent series, a reticulin algorithm[9,10] that has shown promise in the diagnosis of carcinoma remains to be validated in additional series.

To complicate the matter, intratumoral morphologic, proliferative, and molecular heterogeneity has been recognized in these neoplasms. Microscopic regions with low-grade proliferative features can be encountered in high-grade ACCs, and low-grade ACCs can contain areas indistinguishable from adenomas.[1,5] Furthermore, recent observations also suggest the possibility of adenoma-carcinoma progression in some adrenal cortical neoplasms.[1,6,17,18] These issues are clinically relevant with increasing detection of nonfunctioning adrenal cortical proliferations called "incidentaloma."

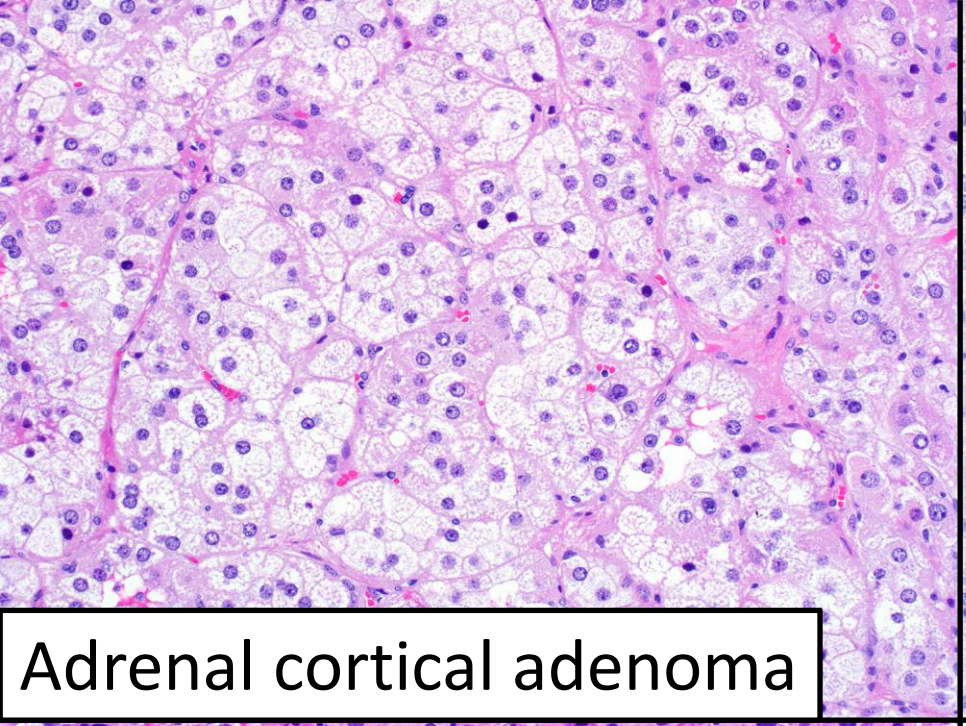
From the *Department of Pathology, University Health Network, †Department of Laboratory Medicine and Pathobiology, University of Toronto; ‡Endocrine Oncology Site Group, The Princess Margaret Cancer Centre, Toronto, ON, Canada; §Department of Pathology, Recep Tayyip Erdogan University, Rize; and ‖Department of Pathology, Ondokuz Mayis University, Samsun, Turkey.

O.M.: concept and design, literature search and writing. O.M. and S.L.A.: critical reviews. O.M., S.L.A., H.G., and M.K.: data collection and/or processing. O.M. and H.G.: analysis and/or interpretation. S.L.A.: financial resources.

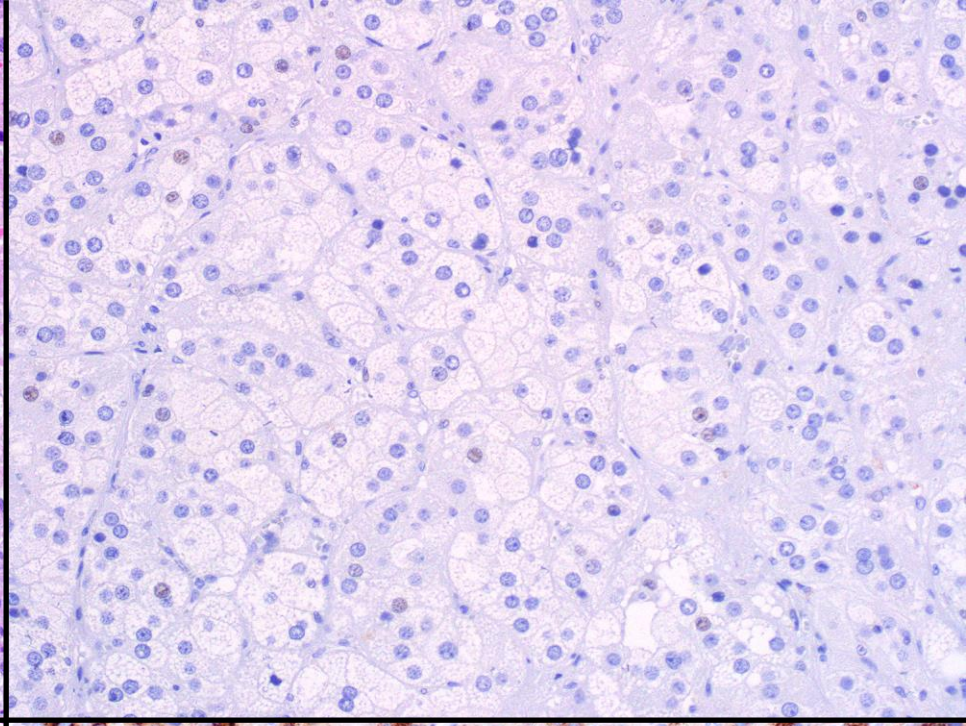
The preliminary results of this study were presented during the endocrine platform presentations at the USCAP meeting in 2014, San Diego, CA.

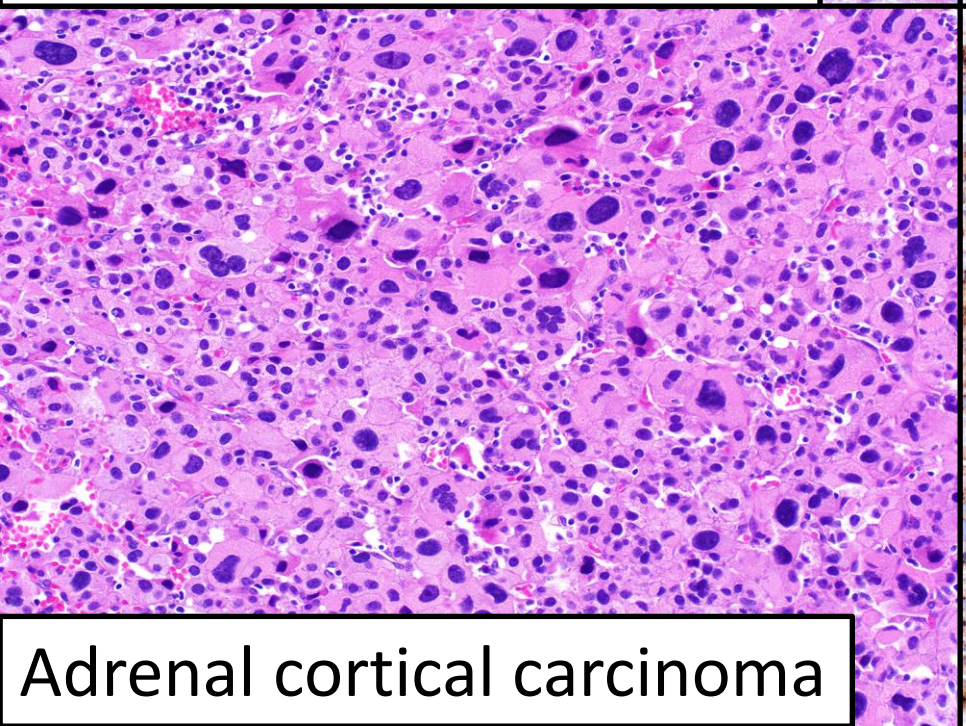
Conflicts of Interest and Source of Funding: The authors have disclosed that they have no significant relationships with, or financial interest in, any commercial companies pertaining to this article.

Correspondence: Ozgur Mete, MD, FRCPC, Department of Pathology, University Health Network, 200 Elizabeth Street, 11th floor, Toronto, ON, Canada M5G 2C4 (e-mail: ozgur.mete2@uhn.ca).

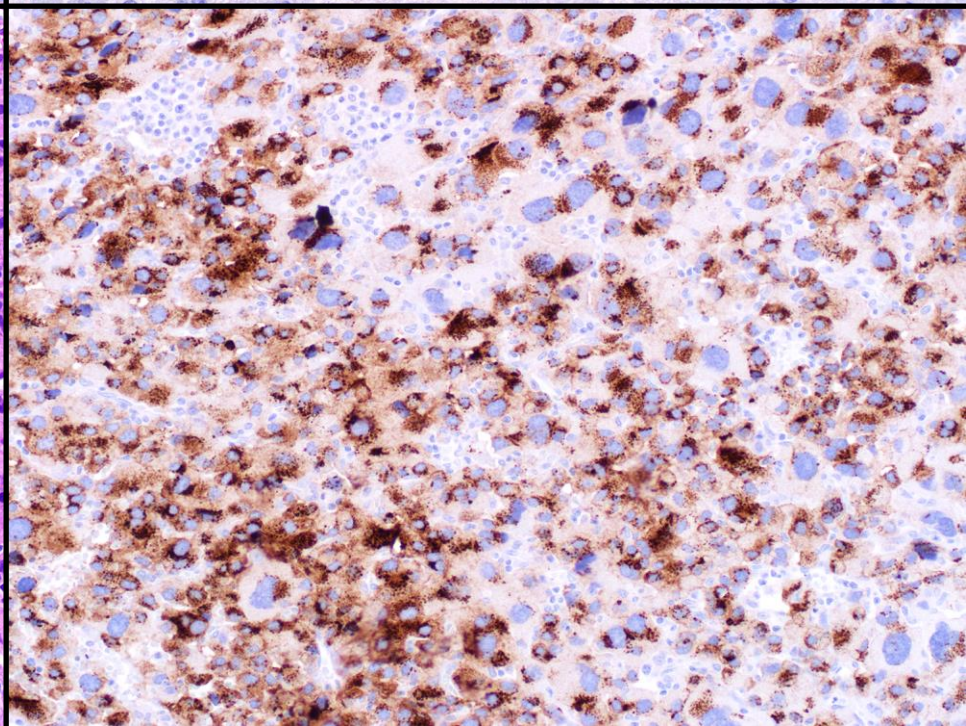


Adrenal cortical adenoma



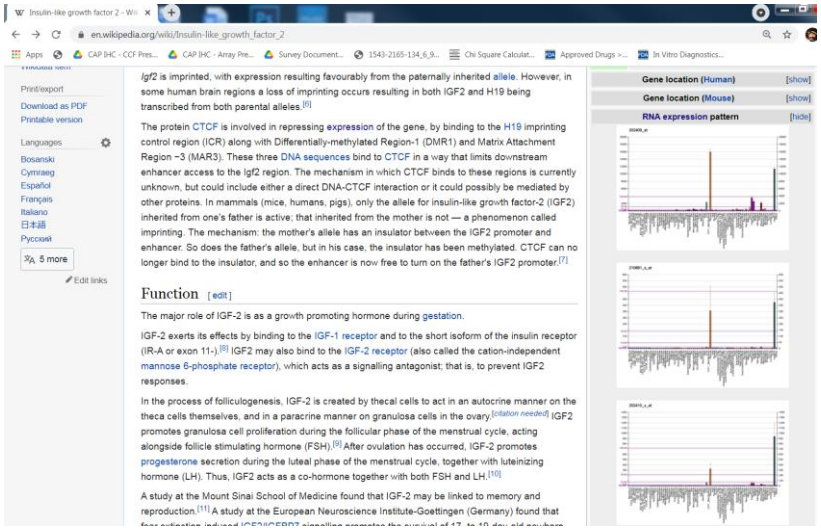


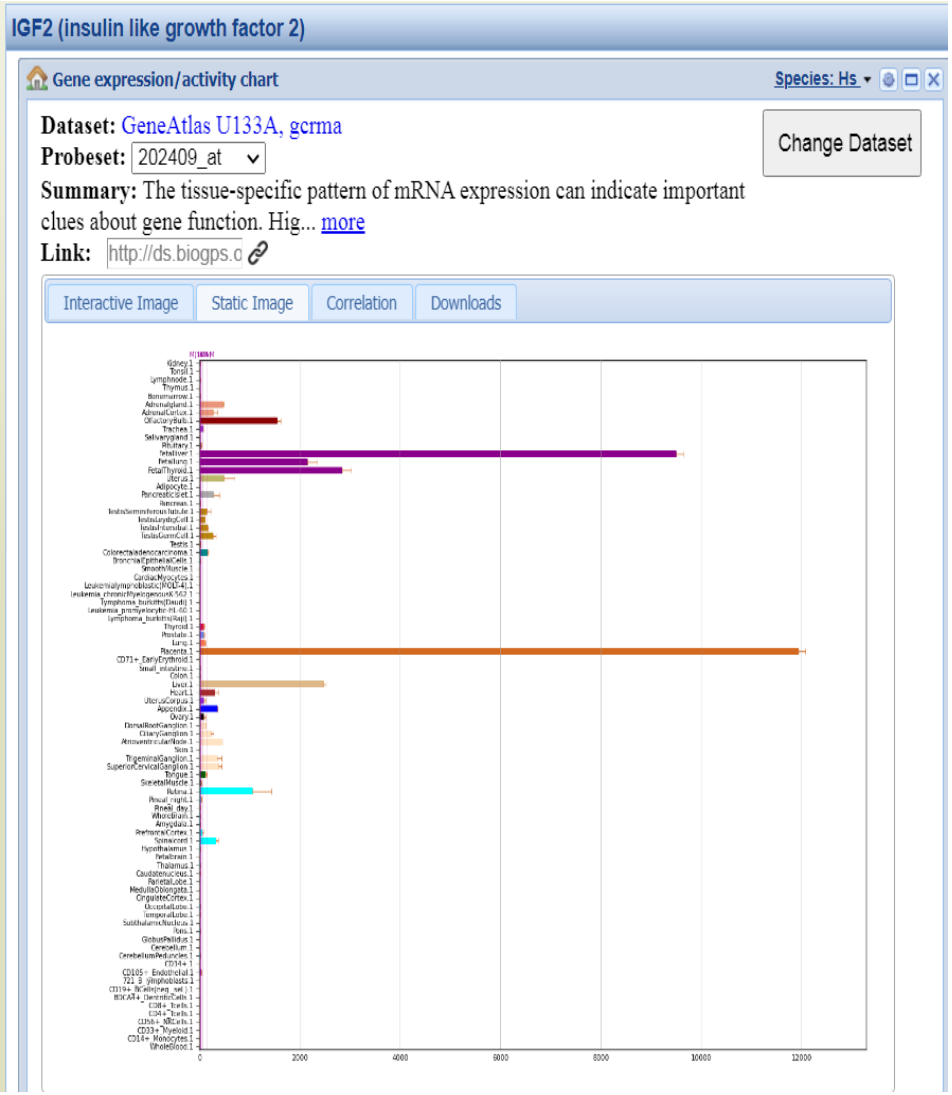
Adrenal cortical carcinoma



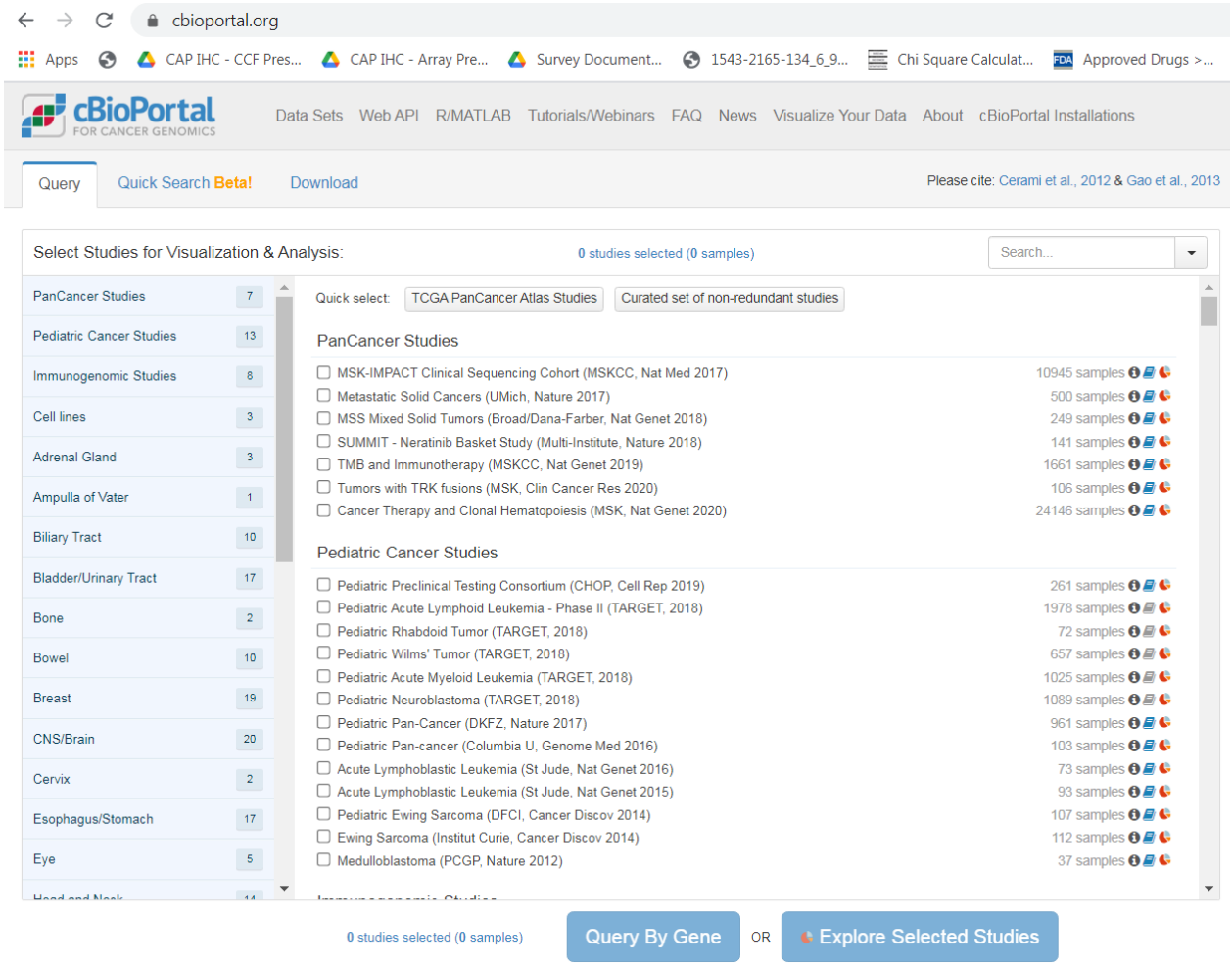
How to visualize gene expression

- GeneCards
  - GTEx
  - Illumina
  - BioGPS
- Wikipedia☺



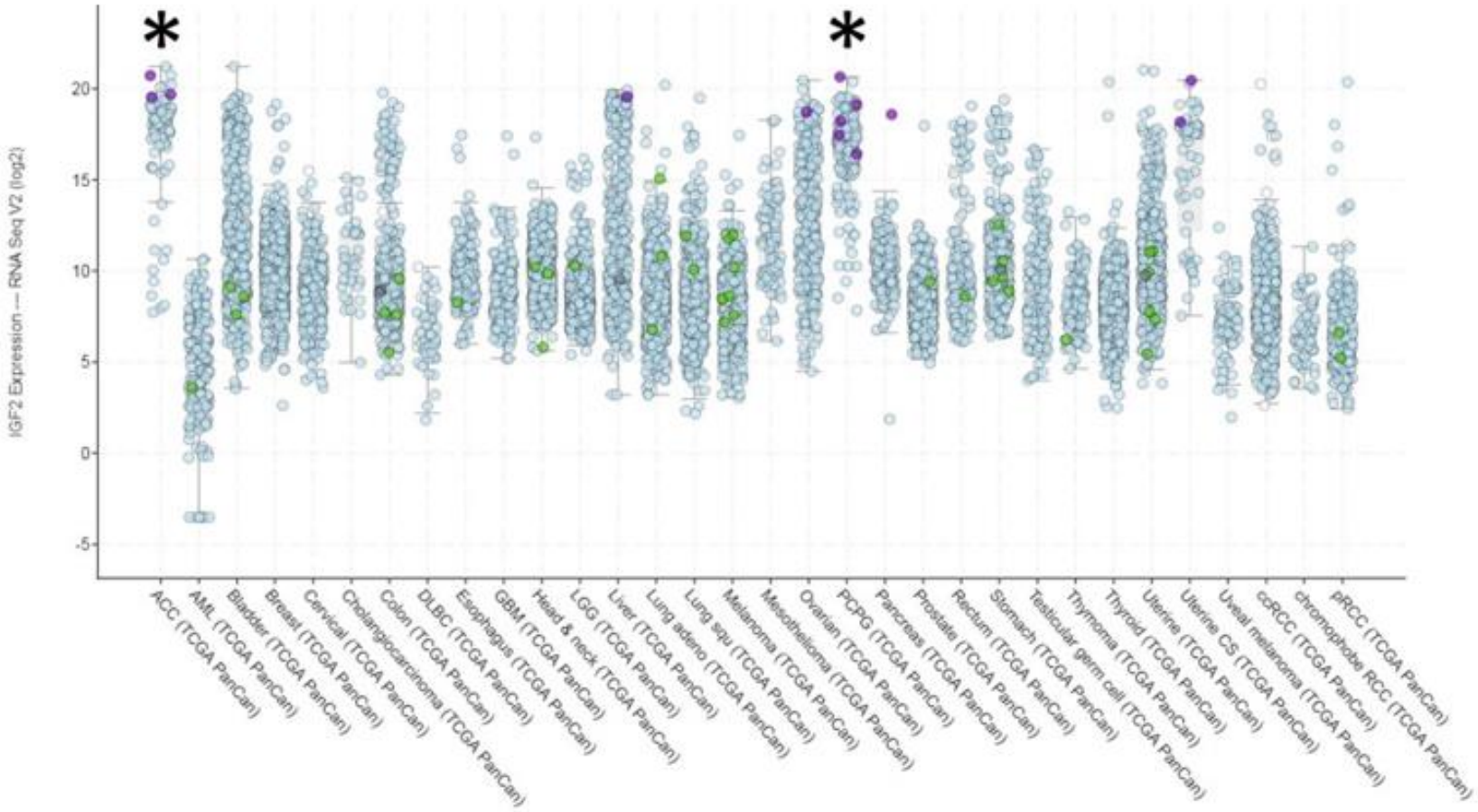


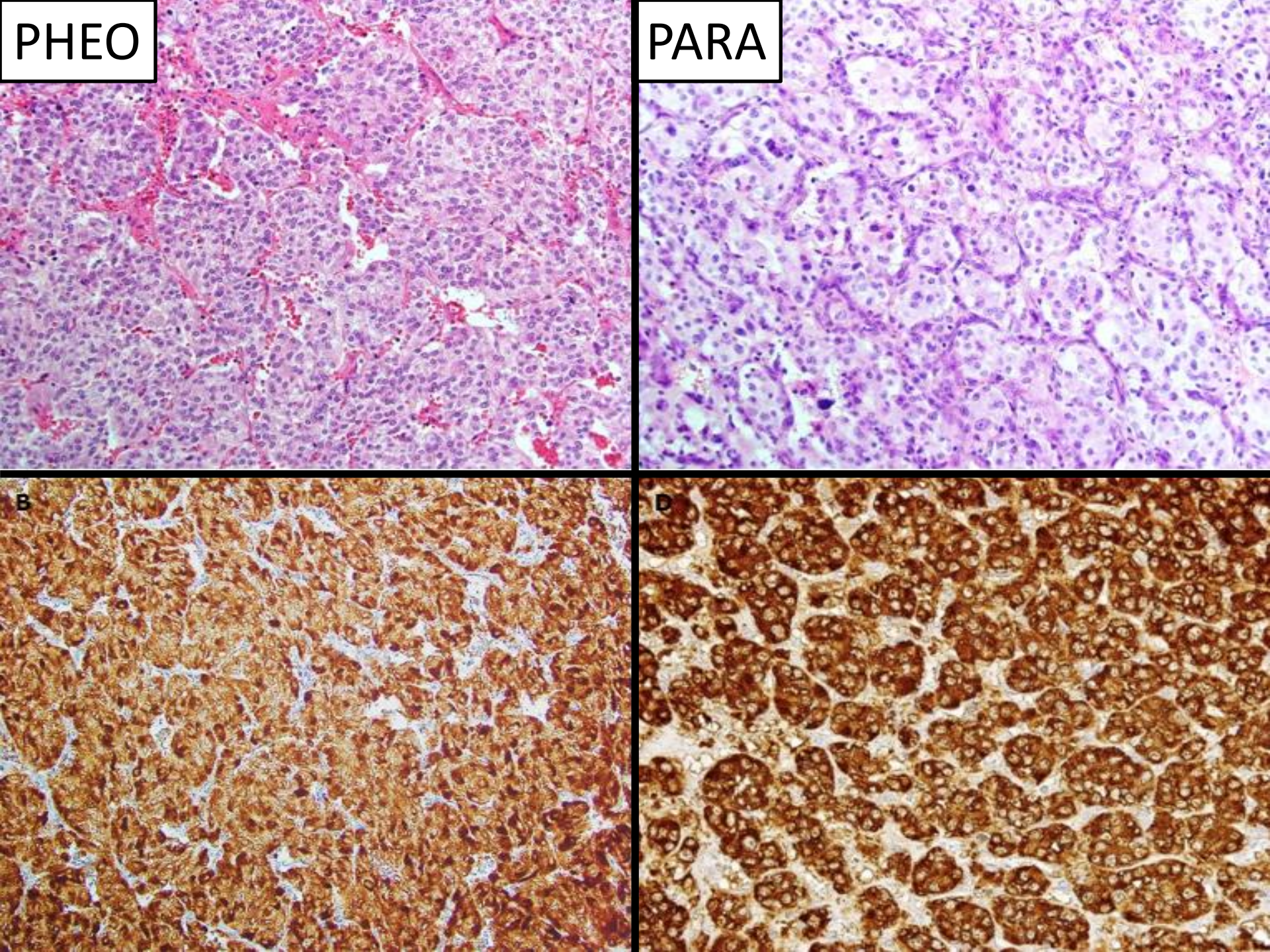
cBioPortal: How I Visualize Genetic & Gene Expression Profiling Data in Cancer

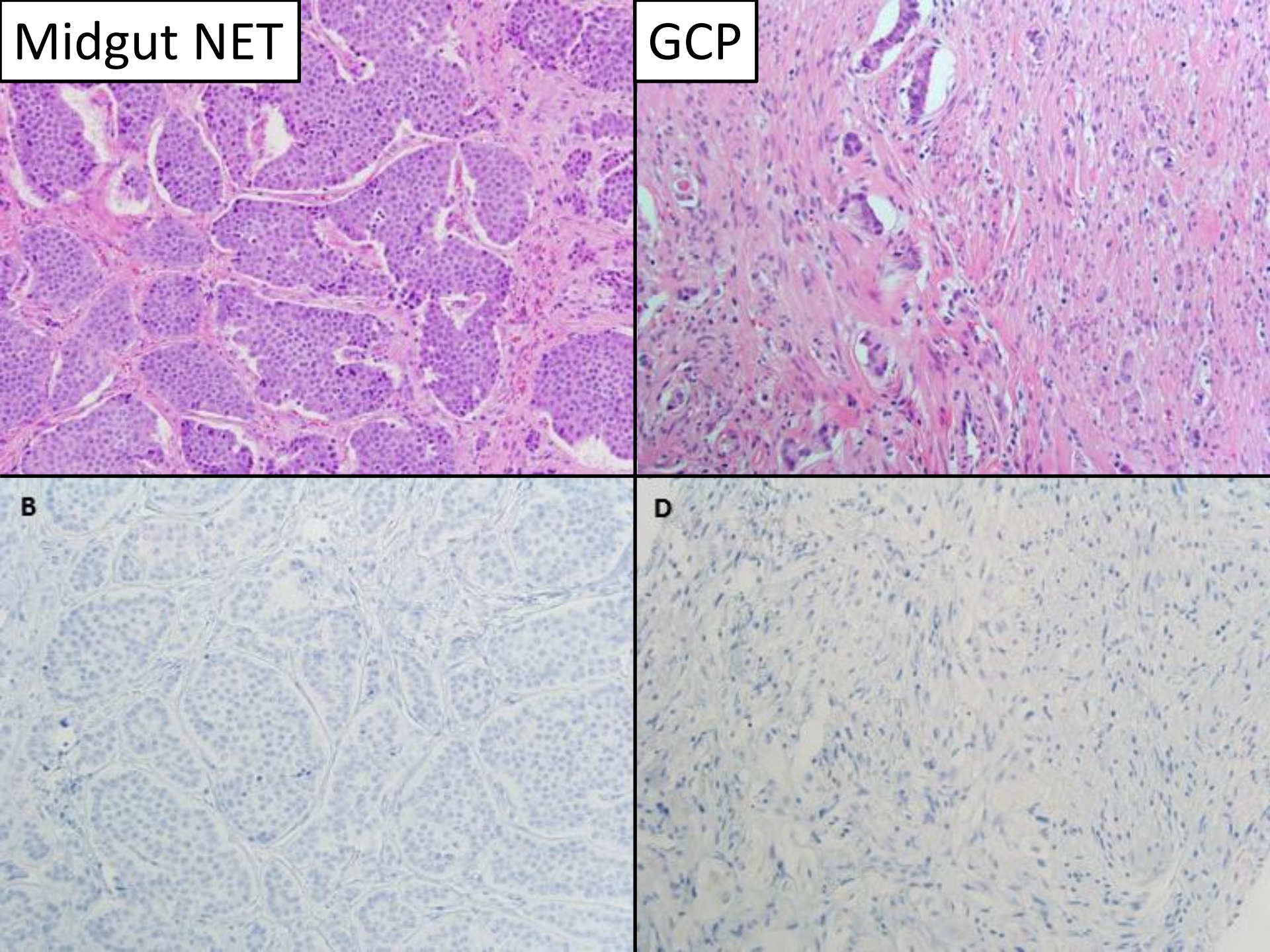


The cBioPortal for Cancer Genomics was originally developed at Memorial Sloan Kettering Cancer Center (MSK). The public cBioPortal site is hosted by the Center for Molecular Oncology at MSK. The cBioPortal software is now available under an open source license via GitHub. The software is now developed and maintained by a multi-institutional team, consisting of MSK, the Dana Farber Cancer Institute, Princess Margaret Cancer Centre in Toronto, Children's Hospital of Philadelphia, The Hyve in the Netherlands, and Bilkent University in Ankara, Turkey.

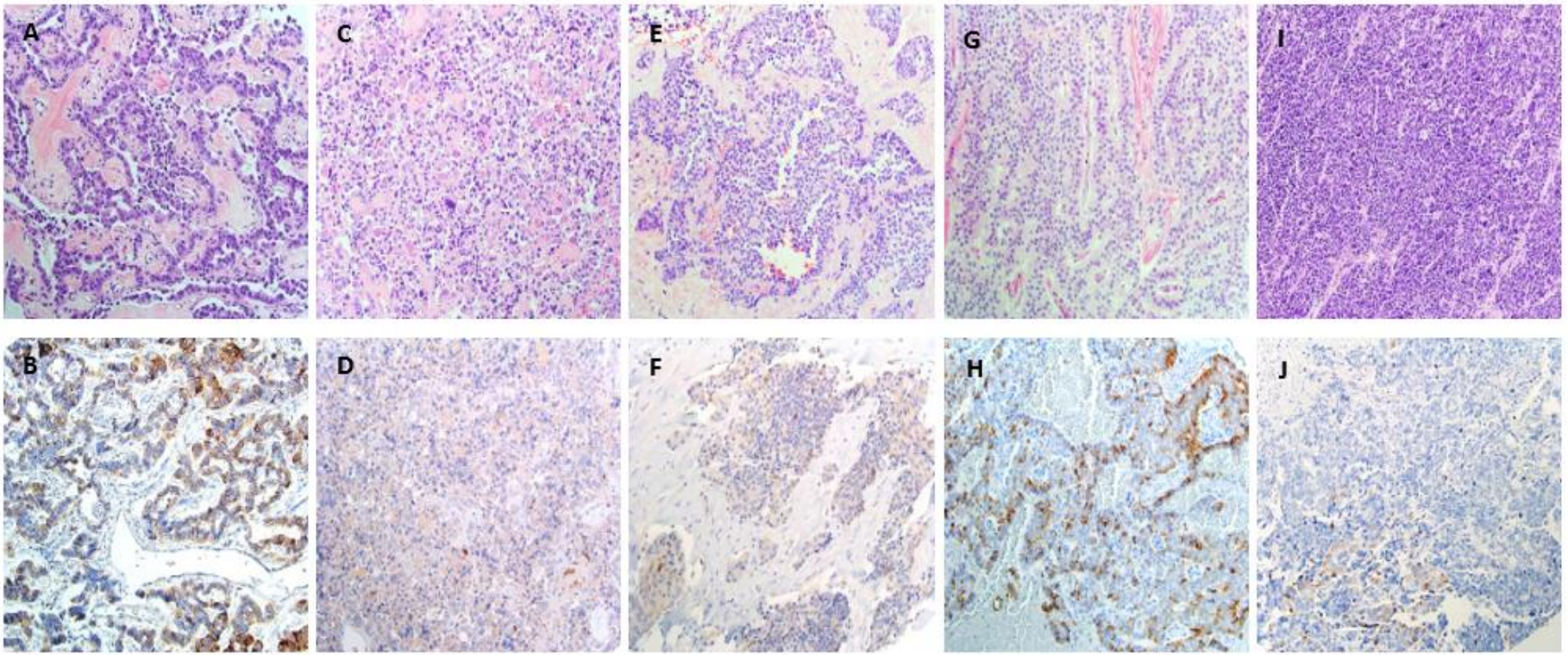
Pan-Cancer IGF2 Gene Expression



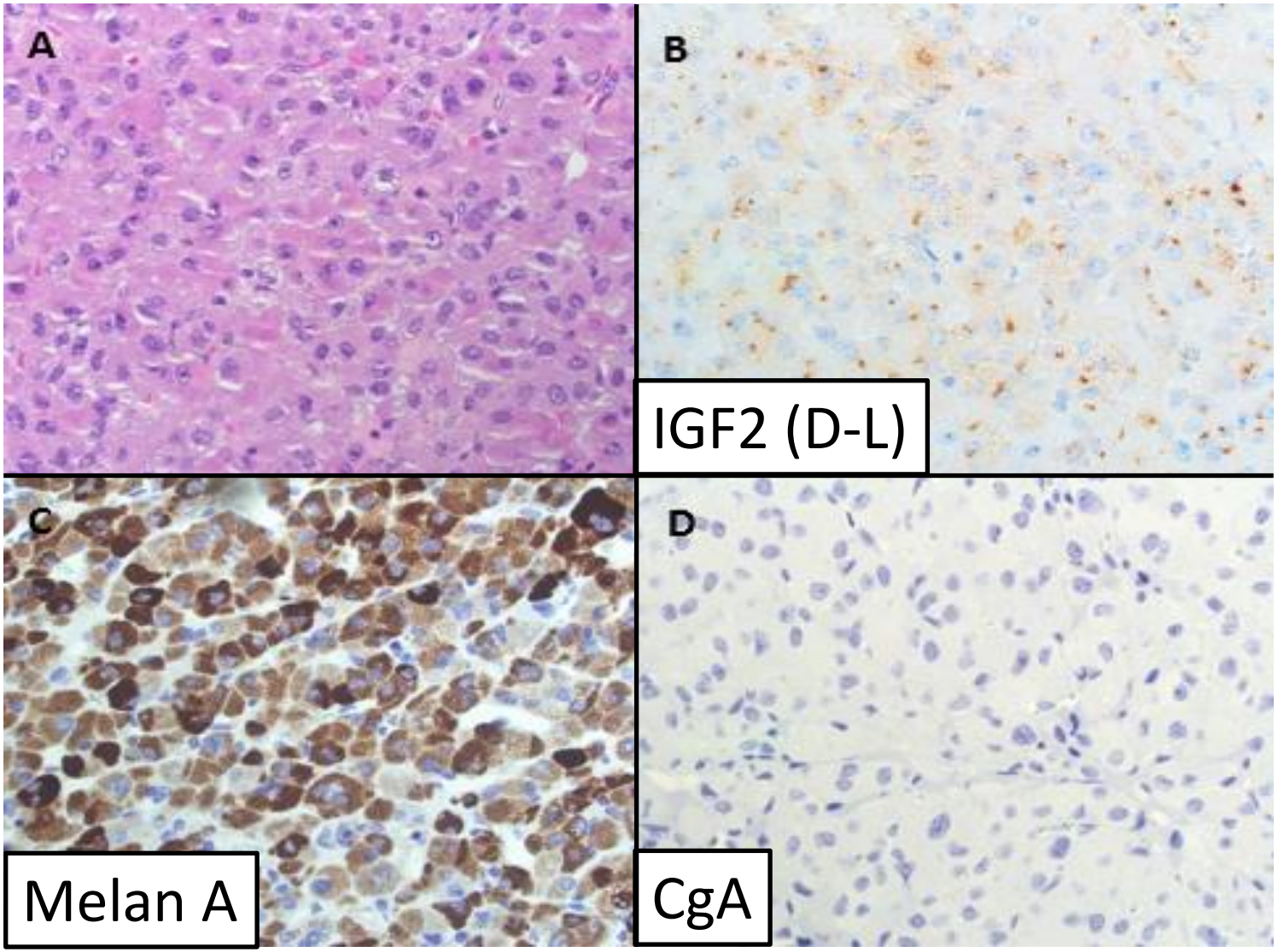

PHEO
PARA
B
D


Midgut NET
GCP
B
D

The “Floridly” Positive NET/NECs



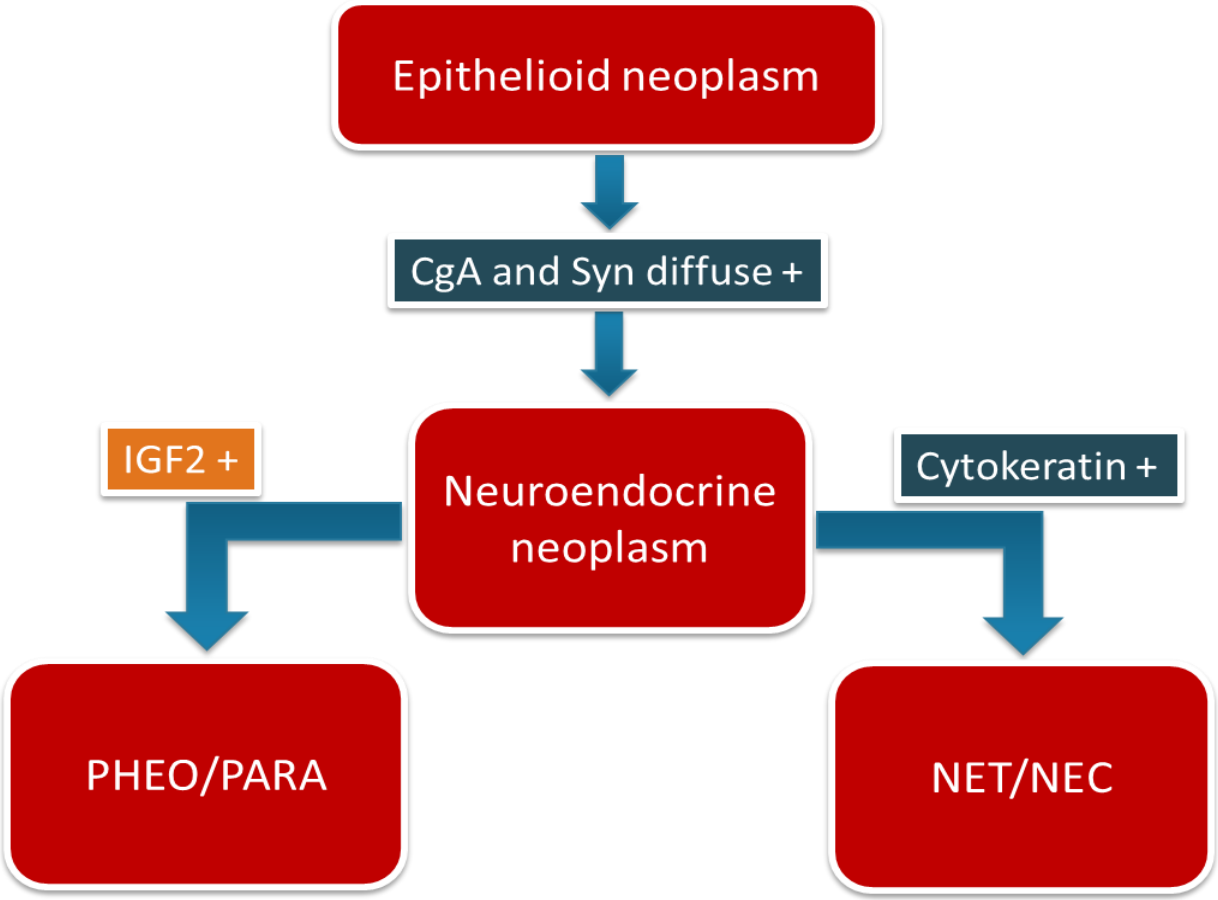
We Even Reclassified 2 PHEOs→ACC



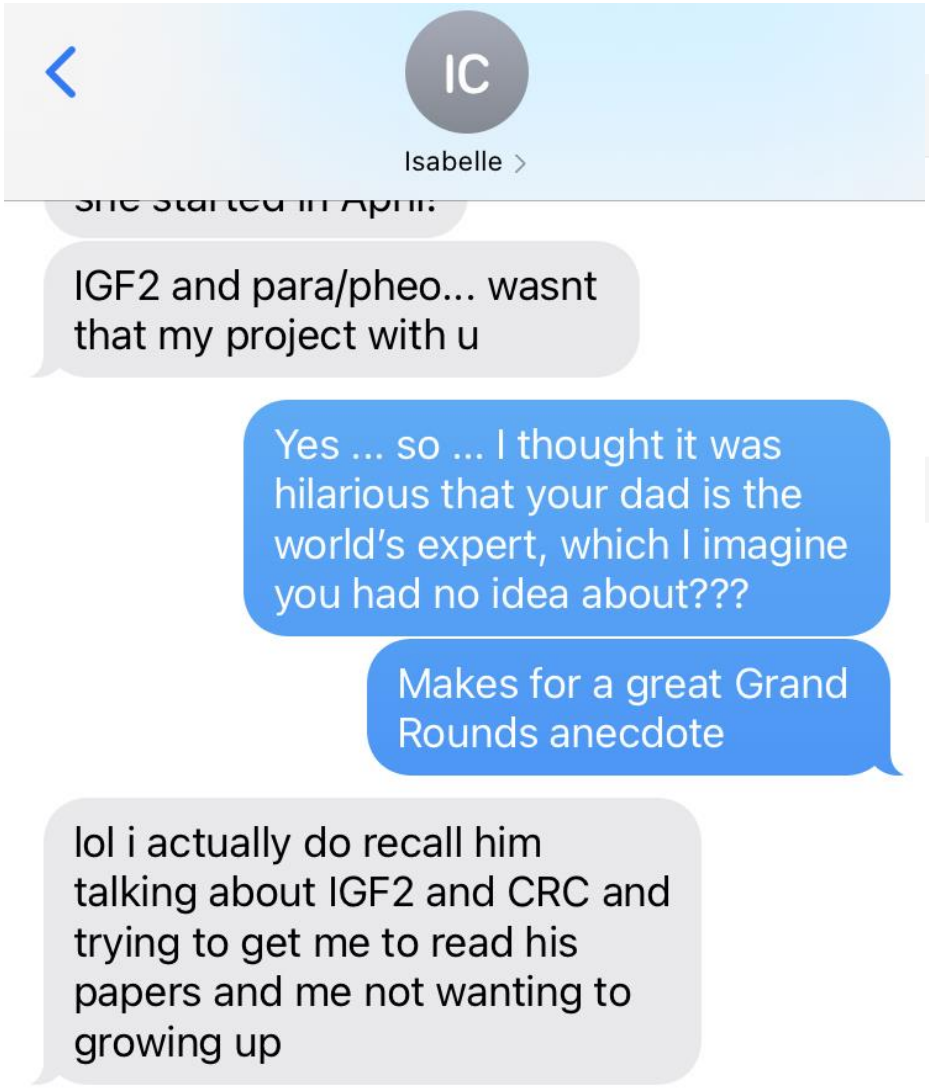
IGF2 Expression in Neuroendocrine Neoplasms

Tumor Type	% Positive	Mean H-score (Median)
Pheochromocytoma	100%	270 (300)
Paraganglioma	87%	235 (270)
Pancreas NET	19%	129 (145)
Jejunoileum NET	0%	NA
Medullary thyroid carcinoma	14%	9 (7)
Lung NET	16%	124 (10)
Duodenal NET	14%	13 (13)
Appendiceal NET	80%	22 (20)
NEC	7%	45 (7)
Gangliocytic paraganglioma	0%	NA

Algorithm to distinguish PHEO/PARA vs NET/NEC



Because Life is Full of Wonderful Coincidences



[CANCER RESEARCH 62, 6442–6446, November 15, 2002]

Advances in Brief

Loss of Imprinting in Colorectal Cancer Linked to Hypomethylation of *H19* and *IGF2*[1]

Hengmi Cui, Patrick Onyango, Sheri Brandenburg, Yiqian Wu, Chih-Lin Hsieh, and Andrew P. Feinberg[2]

Institute of Genetic Medicine and Department of Medicine [H. C., P. O., S. B., Y. W., A. P. F.], and Departments of Molecular Biology and Genetics, and Oncology Center [A. P. F.], Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, and Departments of Urology and Biochemistry and Molecular Biology, University of Southern California School of Medicine, Los Angeles, California 90033 [C-L. H.]

Abstract

Epigenetic alterations in human cancers include global DNA hypomethylation, gene hypomethylation and promoter hypermethylation, and loss of imprinting (LOI) of the insulin-like growth factor-II gene (*IGF2*). A mechanism for LOI described previously is hypermethylation of a differentially methylated region (DMR) upstream of the *H19* gene, allowing activation of the normally silent maternal allele of *IGF2*. Here we show that this mechanism does not apply to colorectal cancers, which show hypomethylation of the *H19* DMR as well as a DMR upstream of exon 3 of *IGF2*. This hypomethylation is found in both colorectal cancers and normal mucosa from the same patients, and in cell lines with somatic cell knockout of DNA methyltransferases *DNMT1* and *DNMT3B*. These data suggest that hypomethylation is a mechanism for LOI, that the popular *IGF2-H19* enhancer competition model for *IGF2* imprinting does not apply to the human colon, and that an alternative model for LOI would involve a transcriptional repressor acting on the normally silent maternal allele of *IGF2*.

Introduction

Epigenetic alterations in human cancer, *i.e.*, alterations in the genome other than the DNA sequence itself, were first described in 1983 by Feinberg and Vogelstein (1), who found widespread hypomethylation of genes in CRCs[3] and in premalignant adenomas. Epigenetic abnormalities identified subsequently include global genomic hypomethylation (2), promoter hypermethylation of CpG islands (3, 4), and LOI (5, 6), or loss of the normal parent of origin-dependent gene silencing, affecting at least the genes *IGF2*, *PEG1*, *p73*, and *LIT1* (5–11). LOI of *IGF2* causes overexpression of *IGF2* (12), an important autocrine growth factor in cancer. LOI was first identified in embryonal tumors in childhood, including Wilms' tumor, in which it is the most common molecular alteration (5, 6), as well as rhabdomyosarcoma (13) and hepatoblastoma (14). LOI was also later found in common adult malignancies including ovarian (15), colon (16), lung (17), and bladder cancer (18), as well as chronic myelogenous leukemia (19). In CRC, LOI is particularly important because it is found commonly in both the tumor and normal tissue of patients with CRC, at ~3-fold higher frequency then in patients without colon tumors (16), and, thus, LOI may represent the only common alteration linked to cancer that is found in normal tissue.

In Wilms' tumors, approximately half of tumors appear to arise by an epigenetic mechanism involving LOI rather than genetic alterations involving, for example, *WT1* mutations and LOH, and the tumors with LOI appear in children who develop cancer at a later age, accounting for the bimodal age distribution of Wilms' tumor (12). LOI was linked to increased methylation, because Wilms' tumors with LOI of *IGF2*, *i.e.*, activation of the normally silent maternal allele, show aberrant methylation of the normally unmethylated maternal allele of a DMR upstream of the *H19* gene on the same chromosome (20, 21). This result is consistent with the enhancer competition model for regulation of *H19* imprinting. By this model, *IGF2* and *H19* promoters compete on the same chromosome for a shared enhancer, and access of the maternal *IGF2* allele to this enhancer is blocked by the *H19* DMR when unmethylated, likely because of the insulator activity of CTCF binding to the unmethylated *H19* DMR (Refs. 22–26). Indeed, we observed that in Wilms' tumor, methylation of the maternal *H19* DMR includes CTCF-binding sites (27). These results would suggest that increased or ectopic activity of a DNA methyltransferase might lead to aberrant methylation of the maternal *H19* DMR.

Therefore, we were surprised to observe that HCT116, a CRC line with normal imprinting of *IGF2*, is hypermethylated at *H19* and retains normal imprinting after somatic cell knockout of the maintenance DNA methyltransferase *DNMT1* but loses imprinting after subsequent somatic cell knockout of *DNMT3B* (28), a *de novo* methyltransferase, *i.e.*, that is able to methylate unmethylated sequences and is necessary for normal imprinting (29, 30). This result implies that the loss of methylation, rather than the gain of methylation, causes LOI in CRC. To better determine whether LOI in CRC involves hypomethylation or hypermethylation, we performed genomic sequencing analysis. Our results differ from past studies, and they also suggest a model of *IGF2* imprinting in at least the colon that differs from the conventional view of enhancer competition between *IGF2* and *H19*.

Materials and Methods

Bisulfite Sequencing Analysis. *H19* CTCF binding site 1 (CBS1) was analyzed as described earlier (27); CBS6 corresponds to GenBank nucleotides 7855–8192 (accession no. AF125183) and was analyzed after bisulfite treatment using primers 5′-GAGTTTGGGGGTTTTTGTATAGTAT-3′ and 5′-CTTAAATCCCAAACCATAACACTA-3′, followed by 5′-GTATATGGGTATTTTTGGAGGT-3′ and 5′-CCATAACACTAAAACCCTCAA-3′, both annealing at 55°C. The *IGF2* DMR sequence analyzed corresponds to GenBank nucleotides 631–859 (accession no. Y13633), and was analyzed after bisulfite treatment using primers 5′-GGGAATGTTTATTTATGTATGAAG-3′ and 5′-TAAAAACCTCCTCCACCTCC-3′, annealing at 55°C, followed by 5′-TAATTTATTTAGGGTGGTGTT-3′ and 5′-TCCAAACACCCCACCTTAA-3′, annealing at 50°C. Other conditions are as described earlier (27).

Methyltransferase Activity Analysis. *In vitro* functional analysis was performed using the 293/EBNA1 cell line as described (31) and the pcDNA3Myc vector containing full-length DNMT3B coding sequences, and p220.2 (32) as the assay plasmid. Cotransfected target DNA was digested with the methylation-sensitive restriction endonuclease *Hpa*II, and Southern blot was performed using p220.2 as a probe. All of the transfections were done in duplicate or triplicate for each experiment.

Received 7/9/02; accepted 10/4/02.
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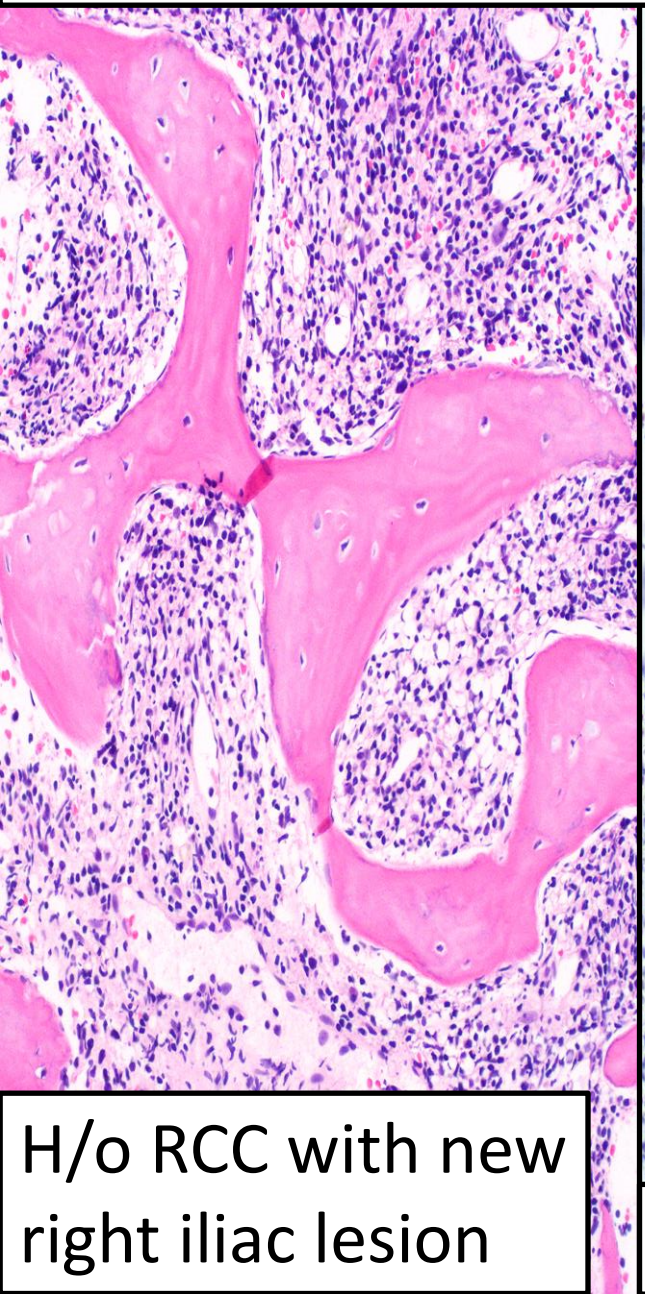
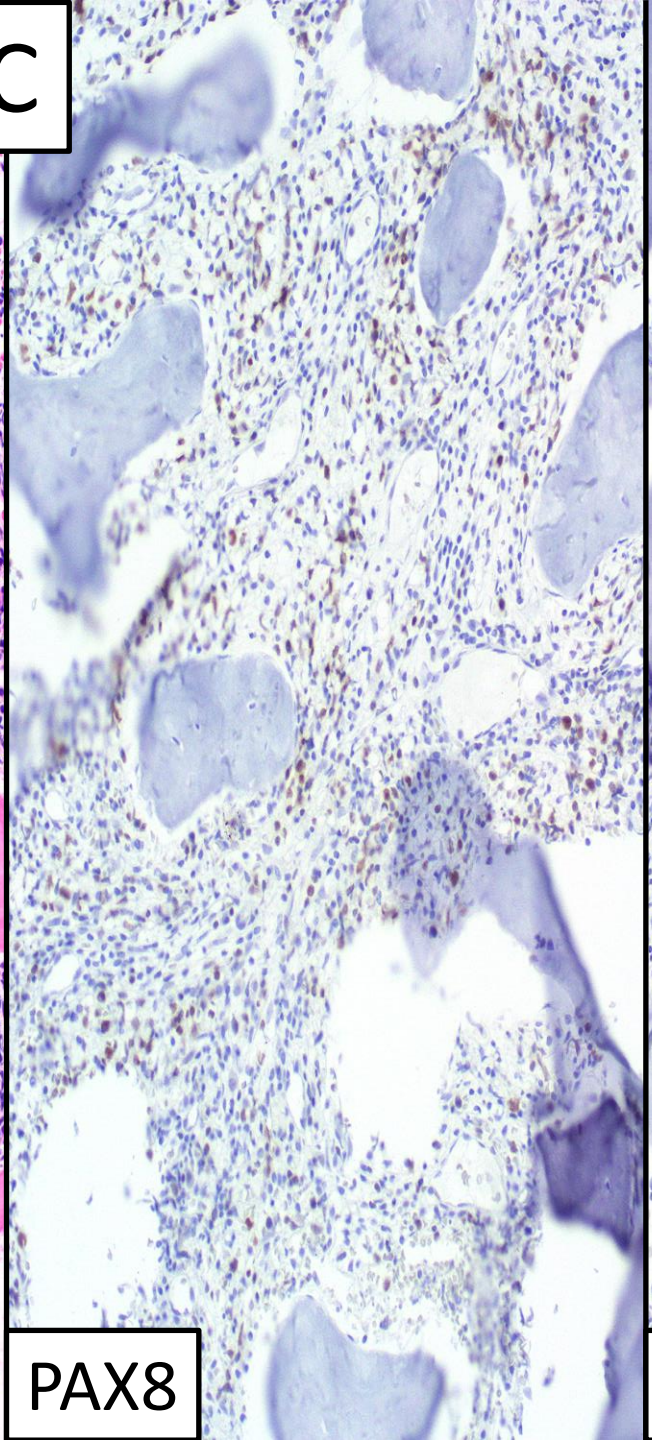
[1] Supported by NIH Grant R01 CA65145 (to A. P. F.)
[2] To whom requests for reprints should be addressed, at Ross 1064, Johns Hopkins University School of Medicine, 720 Rutland Avenue, Baltimore, MD 21205. Phone: (410) 614-3489; Fax: (410) 614-9819; E-mail: afeinberg@jhu.edu.
[3] The abbreviations used are: CRC, colorectal cancer; LOI, loss of imprinting; LOH, loss of heterozygosity; DMR, differentially methylated region.

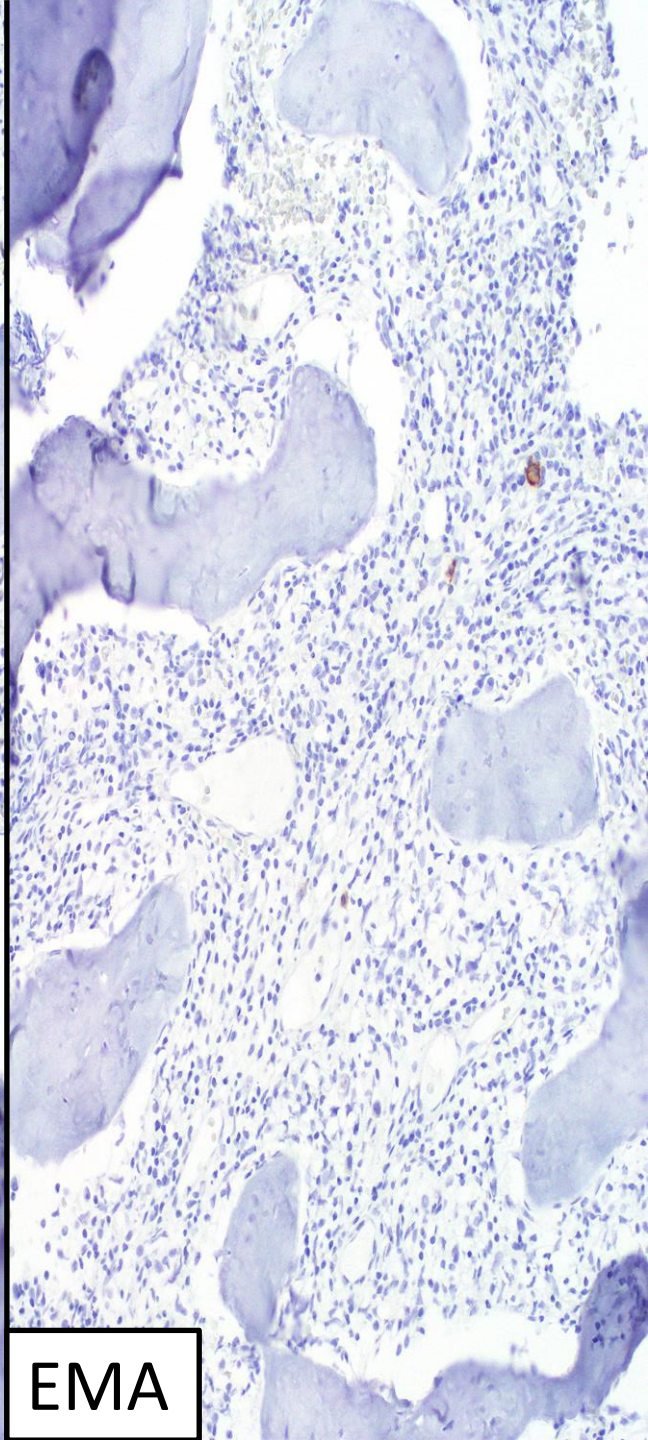
Metastatic RCC



H/o RCC with new right iliac lesion

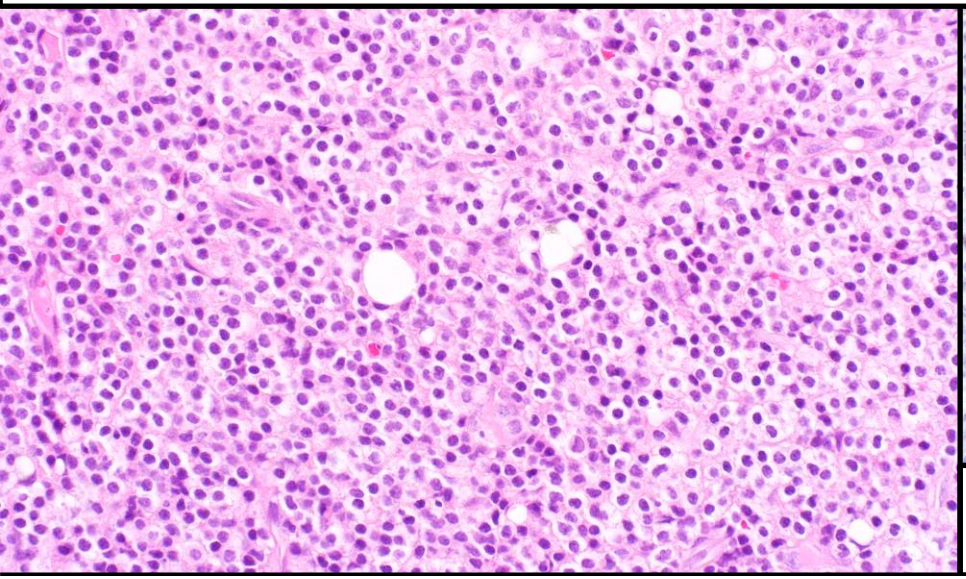


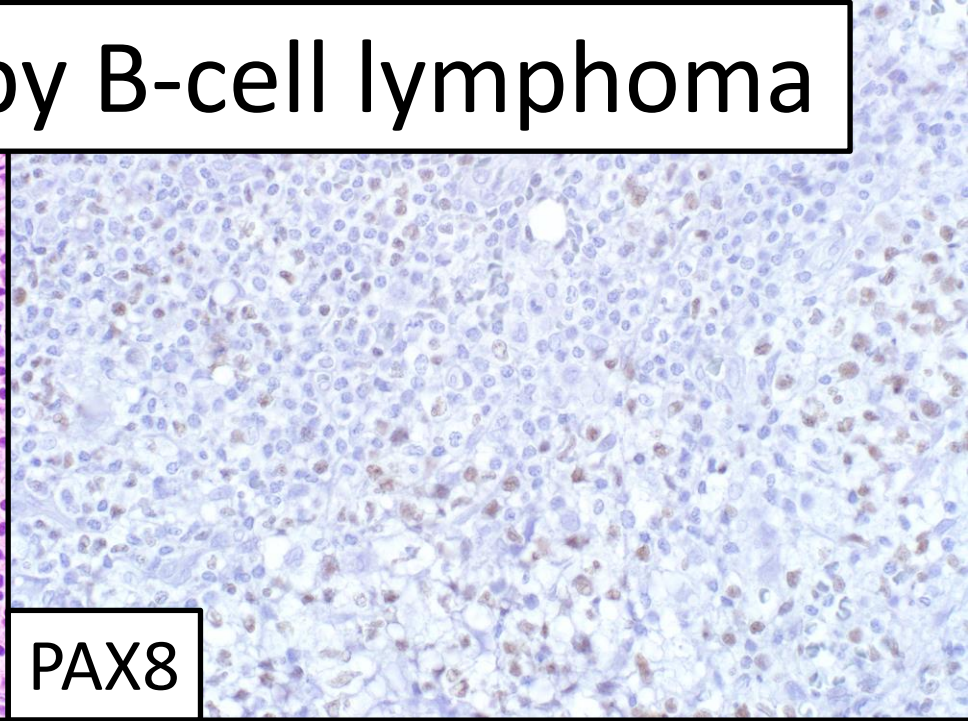
PAX8



EMA

Revised dx: involved by B-cell lymphoma





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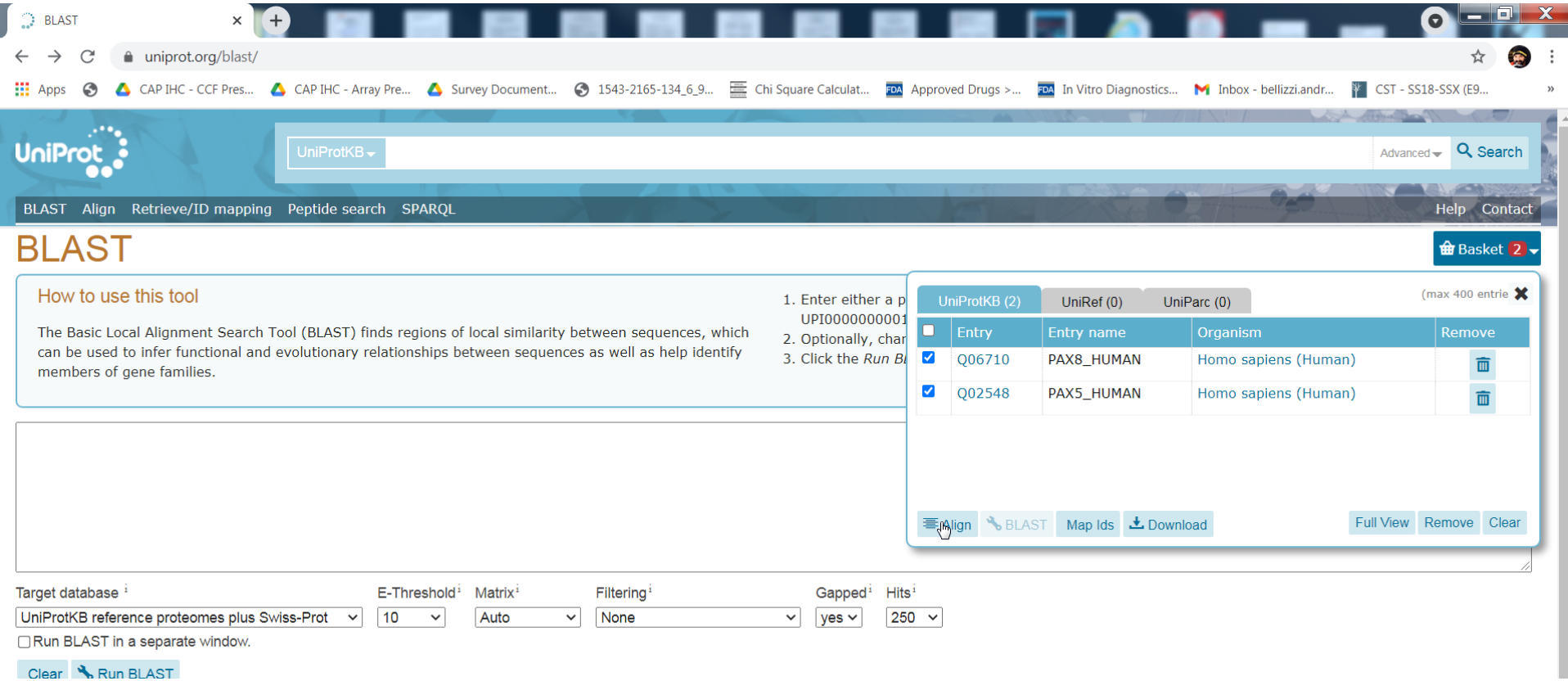
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How to use this tool
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10
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250
Run BLAST in a separate window.
Clear
Run BLAST

PAX8 Fusion Protein

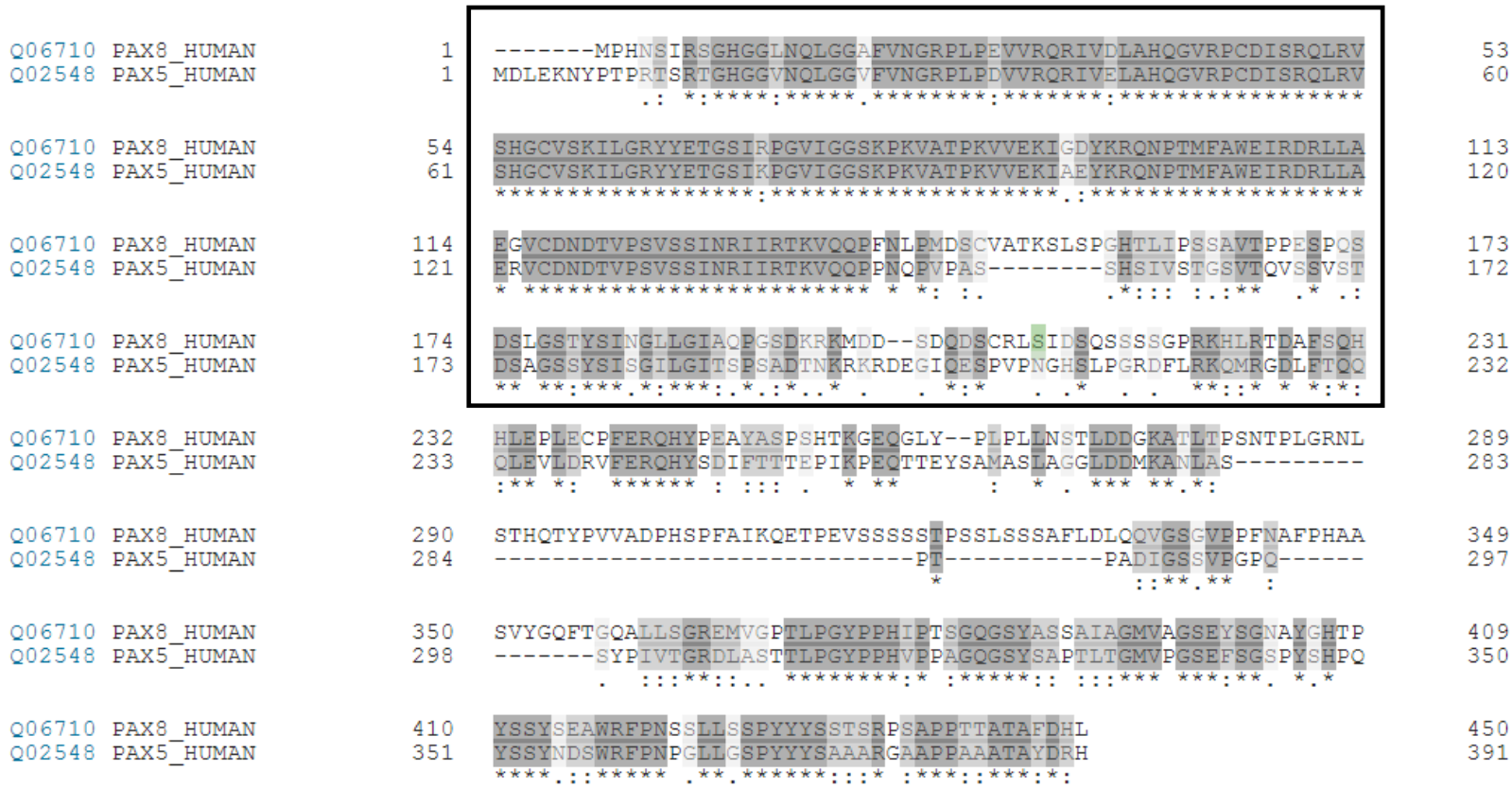
Catalog number: Ag0306

MSDS

Datasheet

Product Name:	human PAX8-GST fusion protein
Catalog No:	Ag0306
Source:	*e coli.*-derived, PGEX-4T
Peptide Sequence:	MPHNSIRSGHGGLNQLGGAFVNGRPLPEVVRQRIVDLAHQGVRPCDISRQLRVSHG CVSKILGRYYETGSIRPGVIGGSKPKVATPKVVEKIGDYKRQNPTMFAWEIRDRLLAEG VCDNDTVPSVSSINRIIRTKVQQPFNLPMDSCVATKSLSPGHTLIPSSAVTPPESPQSD SLGSTYSINGLLGIAQPGSDKRKMDDSDQDSCRLSIDS (1-212 aa encoded by BC001060)

Alignment of PAX8 and PAX5 Proteins



Polyclonal vs Monoclonal PAX8 in Thoracic Pathology

Histopathology

Histopathology 2014, 65, 465–472. DOI: 10.1111/his.12405

Utility of PAX8 mouse monoclonal antibody in the diagnosis of thyroid, thymic, pleural and lung tumours: a comparison with polyclonal PAX8 antibody

Akane Toriyama,[1,2] Taisuke Mori,[1,3] Shigeki Sekine,[1,4] Akihiko Yoshida,[1] Okio Hino[4] & Koji Tsuta[1]

[1]*Division of Pathology and Clinical Laboratories, National Cancer Center Hospital, Tokyo, Japan,* [2]*Department of Pathology, Juntendo University Urayasu Hospital, Chiba, Japan,* [3]*Division of Pathology, National Cancer Center Research Institute, Tokyo, Japan, and* [4]*Department of Pathology and Oncology, Juntendo University School of Medicine, Tokyo, Japan*

Date of submission 26 November 2013
Accepted for publication 28 February 2014
Published online Article Accepted 4 March 2014

Toriyama A, Mori T, Sekine S, Yoshida A, Hino O & Tsuta K (2014) *Histopathology* **65**, 465–472

Utility of PAX8 mouse monoclonal antibody in the diagnosis of thyroid, thymic, pleural and lung tumours: a comparison with polyclonal PAX8 antibody

Aims: The purpose of this study was to compare the immunohistochemical staining profiles of PAX8-polyclonal, PAX8-monoclonal, PAX5-monoclonal and PAX6-monoclonal antibodies in several histological types of primary thoracic and thyroid tumours. In addition, we analysed *PAX8* mRNA expression by using *in-situ* hybridization.

Methods and results: We compared polyclonal PAX8 and monoclonal PAX8, PAX5 and PAX6 antibodies in 962 samples (687 lung carcinomas, 40 malignant pleural mesotheliomas, 138 thymic tumours and 97 thyroid tumours) using the tissue microarray technique. Among thyroid tumours, the monoclonal and polyclonal PAX8 antibodies showed a high positive rate (98.0%). Of 167 polyclonal PAX8 antibody-positive tumours, except for thyroid tumours, 54 cases tested positive for PAX5 and/or PAX6 (31 lung carcinomas and 23 thymic tumours). No *PAX8* mRNA expression was detected using RNAscope (*in-situ* hybridization technique) other than in thyroid tumours. A portion of polyclonal PAX8 antibody-positive tumours showed cross-reactivity for PAX5 or PAX6 protein.

Conclusions: Monoclonal PAX8 antibody showed high specificity to thyroid tumours and was superior to the polyclonal antibody.

Keywords: cross-reactivity, immunohistochemistry, PAX8, thoracic tumours

Introduction

The lung is the most common site of metastasis for malignant tumours. The distinction of primary lung tumour from metastatic tumour is important, because the treatment modalities and prognosis for these two lesions are quite different. When lung tumours present with typical morphology the diagnosis is straightforward, and immunohistochemical staining is not necessary. However, poorly differentiated tumours are sometimes more challenging. Immunostaining for thyroid transcription factor-1 (TTF-1) is a useful positive marker for confirming adenocarcinoma of a unknown primary site as having a pulmonary origin. However, staining for TTF-1, as the name implies, is also positive in thyroid tumours.

The paired box transcription factor PAX8 is a nephric-lineage transcription factor. In human tissues, it is expressed in various normal tissues, such

Address for correspondence: K Tsuta, Division of Pathology and clinical Laboratories, National Cancer Center Hospital, 1-1 Tsukiji 5-chome, Chuo-ku, Tokyo 104-0045, Japan. e-mail: ktsuta@ncc.go.jp

Table 1. Positive cases stained with polyclonal PAX8, monoclonal PAX8, PAX5, and PAX6 antibodies

Tumour types	Staining pattern in neoplastic cells, no. (%)			
	PAX8 polyclonal	PAX8 monoclonal	PAX5	PAX6
Lung tumours				
Adenocarcinoma (n = 253)	6 (2.4)	0 (0)	0 (0)	26 (10.3)
Squamous cell carcinoma (n = 158)	3 (1.9)	0 (0)	0 (0)	29 (18.4)
Large cell neuroendocrine carcinoma (n = 106)	17 (16.0)	0 (0)	7 (6.6)	20 (18.9)
Small cell carcinoma (n = 67)	27 (40.3)	0 (0)	16 (23.9)	20 (29.9)
Carcinoid tumour (n = 51)	0 (0)	0 (0)	1 (2.0)	7 (13.7)
Pleomorphic carcinoma (n = 41)	0 (0)	0 (0)	0 (0)	2 (4.9)
Large cell carcinoma (n = 11)	2 (18.1)	0 (0)	0 (0)	3 (27.3)
Malignant pleural mesothelioma (n = 40)	0 (0)	0 (0)	0 (0)	5 (12.5)
Thymic tumours				
Thymoma (n = 102)	91 (89.2)	0 (0)	0 (0)	16 (15.7)
Thymic carcinoma (n = 36)	21 (58.3)	0 (0)	7 (19.4)	4 (11.1)
Thyroid tumours				
Papillary carcinoma (n = 80)	79 (98.8)	78 (97.5)	0 (0)	0 (0)
Follicular carcinoma (n = 7)	7 (100)	7 (100)	0 (0)	1 (14.3)
Undifferentiated carcinoma (n = 5)	4 (80.0)	5 (100)	0 (0)	2 (40.0)
Follicular adenoma (n = 5)	5 (100)	5 (100)	0 (0)	0 (0)

ISL1 and PAX6

Insulin gene enhancer binding protein Isl-1 is a member of a novel class of proteins containing both a homeo- and a Cys–His domain

Olof Karlsson, Stefan Thor, Torbjörn Norberg, Helena Ohlsson & Thomas Edlund*

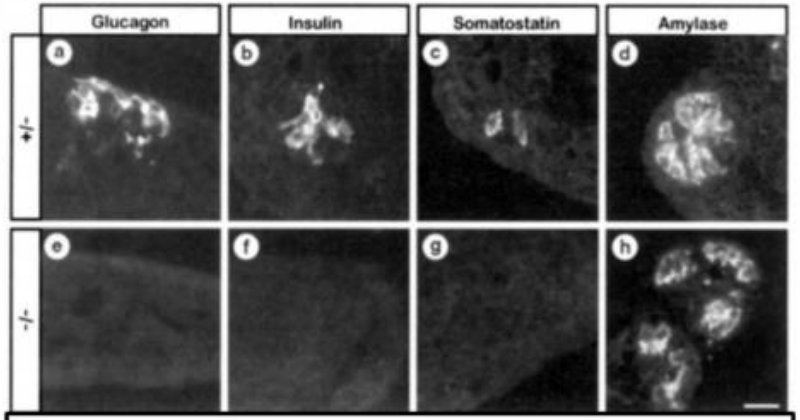
Department of Microbiology, University of Umeå, S-901 87 Umeå, Sweden

NATURE · VOL 344 · 26 APRIL 1990

Independent requirement for ISL1 in formation of pancreatic mesenchyme and islet cells

Ulf Ahlgren*, Samuel L. Pfaff†‡, Thomas M. Jessell†, Thomas Edlund* & Helena Edlund*

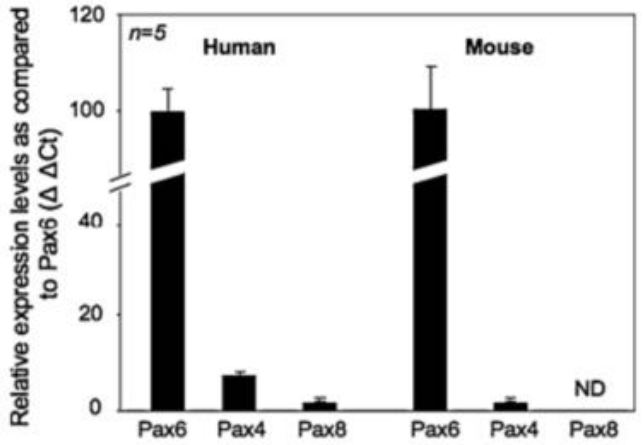
NATURE | VOL 385 | 16 JANUARY 1997

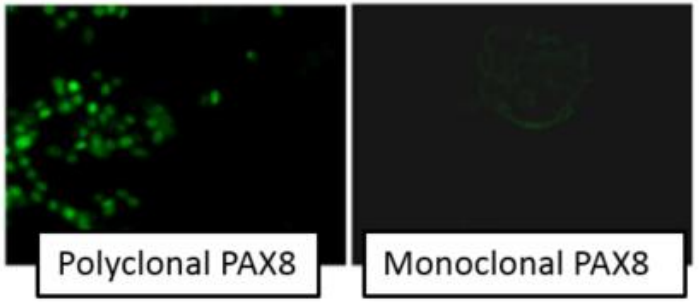


ISL1 homozygous knockout fails to develop islets

PAX8 Is Expressed in Pancreatic Well-Differentiated Neuroendocrine Tumors and in Extrapancreatic Poorly Differentiated Neuroendocrine Carcinomas in Fine-Needle Aspiration Biopsy Specimens

Claudia M. Haynes, MD[1]; Ankur R. Sangoi, MD[1,2]; and Reetesh K. Pai, MD[1]





Lorenzo PI, et al. *Histochem Cell Bio*. 2011;136:595-607.

Extensive Evaluation of Immunohistochemistry to Assign Site of Origin in Well-Differentiated Neuroendocrine Tumors: A Study of 10 Markers in 265 Tumors

Expression of Well-Vetted Markers					
	TTF-1	CDX2	pPAX8	PAX6	ISL1
Lung (n=20)	35	5	0	0	0
Stomach (n=14)	0	7	0	0	7
Duodenum (n=22)	0	48	5	62	82
Pancreas primary (n=57)	0	14	2	79	91
Pancreas metastatic (n=13)	0	0	0	69	85
Jejunoileum primary (n=66)	0	97	0	0	2
Jejunoileum metastatic (n=41)	0	83	0	0	2
Appendix (n=15)	0	100	0	20	47
Rectum (n=17)	0	12	0	56	87

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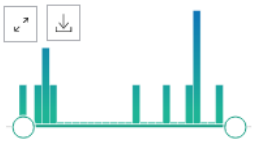

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1 Cite Share

Differential transactivation of the upstream aggrecan enhancer regulated by **PAX1**/9 depends on SOX9-driven transactivation.

Takimoto A, Kokubu C, Watanabe H, Sakuma T, Yamamoto T, Kondoh G, Hiraki Y, Shukunami C.

Sci Rep. 2019 Mar 14;9(1):4605. doi: 10.1038/s41598-019-40810-4.

PMID: 30872687 Free PMC article.

Significant Acan upregulation was observed during chondrification of **Pax1**-silenced AF cells, while, Acan was significantly downregulated by persistent expression of **Pax1** in cartilage. ...Coimmunoprecipitation revealed the physical interaction of **Pax1** with SOX ...

2 Cite Share

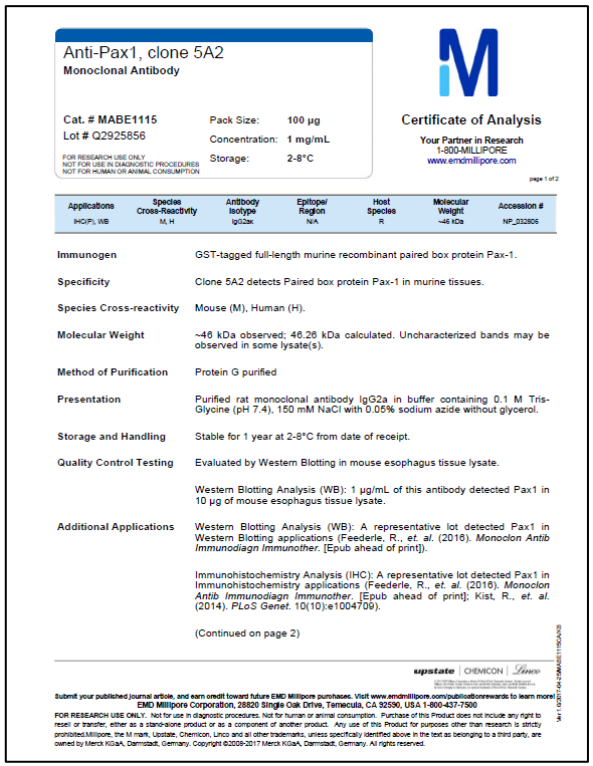
Generation of **Pax1**/**PAX1**-Specific Monoclonal **Antibodies**.

Feederle R, Gerber JK, Middleton A, Northrup E, Kist R, Kremmer E, Peters H.

Monoclon Antib Immunodiagn Immunother. 2016 Oct;35(5):259-262. doi: 10.1089/mab.2016.0029. Epub 2016 Oct 5.

PMID: 27705080

To provide a reagent for reproducible detection of **Pax1** expression, we have generated rat monoclonal **antibodies** (MAbs) against the murine **Pax1** protein. ...The anti-**Pax1** MAbs provide a reliable reagent for reproducible **Pax1**/**PAX1** protein ex ...



Anti-Pax1, clone 5A2
Monoclonal Antibody

Cat. # MABE1115
Lot # Q2925856

Pack Size: 100 µg
Concentration: 1 mg/mL
Storage: 2-8°C

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Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/ Region	Host Species	Molecular Weight	Accession #
(HCP), WB	M, H	IgG2a κ	N/A	R	~46 kDa	NP_032805

Immunogen	GST-tagged full-length murine recombinant paired box protein Pax-1.
Specificity	Clone 5A2 detects Paired box protein Pax-1 in murine tissues.
Species Cross-reactivity	Mouse (M), Human (H).
Molecular Weight	~46 kDa observed; 46.26 kDa calculated. Uncharacterized bands may be observed in some lysate(s).
Method of Purification	Protein G purified
Presentation	Purified rat monoclonal antibody IgG2a in buffer containing 0.1 M Tris-Glycine (pH 7.4), 150 mM NaCl with 0.05% sodium azide without glycerol.
Storage and Handling	Stable for 1 year at 2-8°C from date of receipt.
Quality Control Testing	Evaluated by Western Blotting in mouse esophagus tissue lysate. Western Blotting Analysis (WB): 1 µg/mL of this antibody detected Pax1 in 10 µg of mouse esophagus tissue lysate.
Additional Applications	Western Blotting Analysis (WB): A representative lot detected Pax1 in Western Blotting applications (Feederle, R., *et. al.* (2016). *Monoclon Antib Immunodiagn Immunother.* [Epub ahead of print]). Immunohistochemistry Analysis (IHC): A representative lot detected Pax1 in Immunohistochemistry applications (Feederle, R., *et. al.* (2016). *Monoclon Antib Immunodiagn Immunother.* [Epub ahead of print]; Kist, R., *et. al.* (2014). *PLoS Genet.* 10(10):e1004709).

(Continued on page 2)

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GenePaint

Home of High Resolution Gene Expression Data

About Genepaint

GenePaint is a digital atlas of gene expression patterns in various tissues and species with strong focus on mouse embryos. Expression patterns are determined by non-radioactive *in situ* hybridization on serial tissue sections.

The database is gene-centric and entries can be searched either by gene name, accession number, sequence homology or site of expression. The website features a "virtual microscope" that enables zooming into images down to cellular resolution.

We acknowledge the contribution of the EURExpress consortium.

TUTORIALS

Search and Results

How to search and use the results page

Selection

Manage and share multiple genepaint sets

Viewer

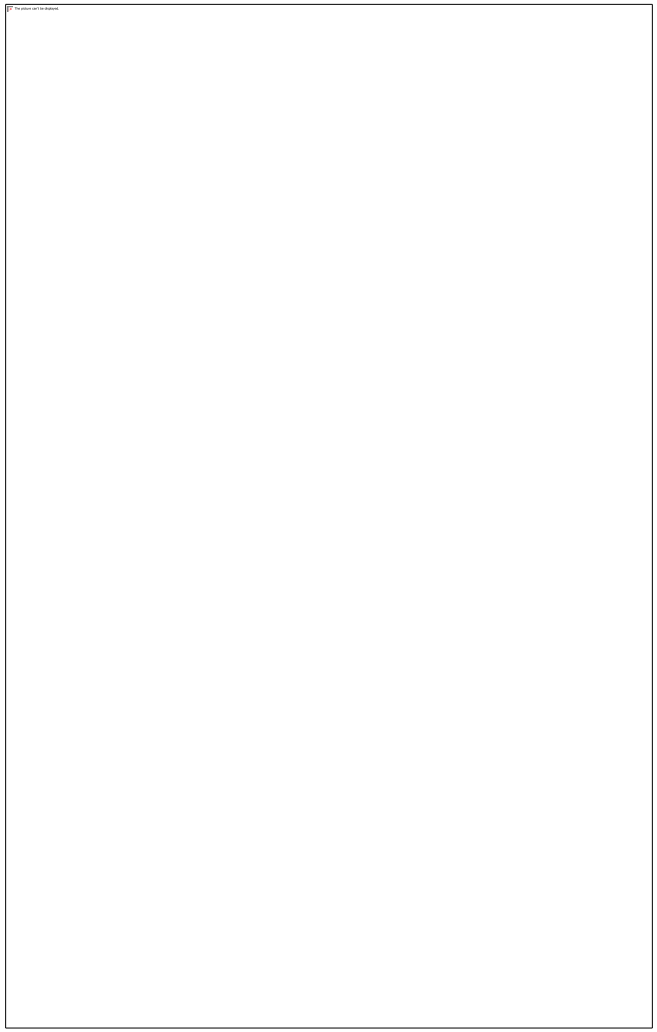
How to use the Viewer

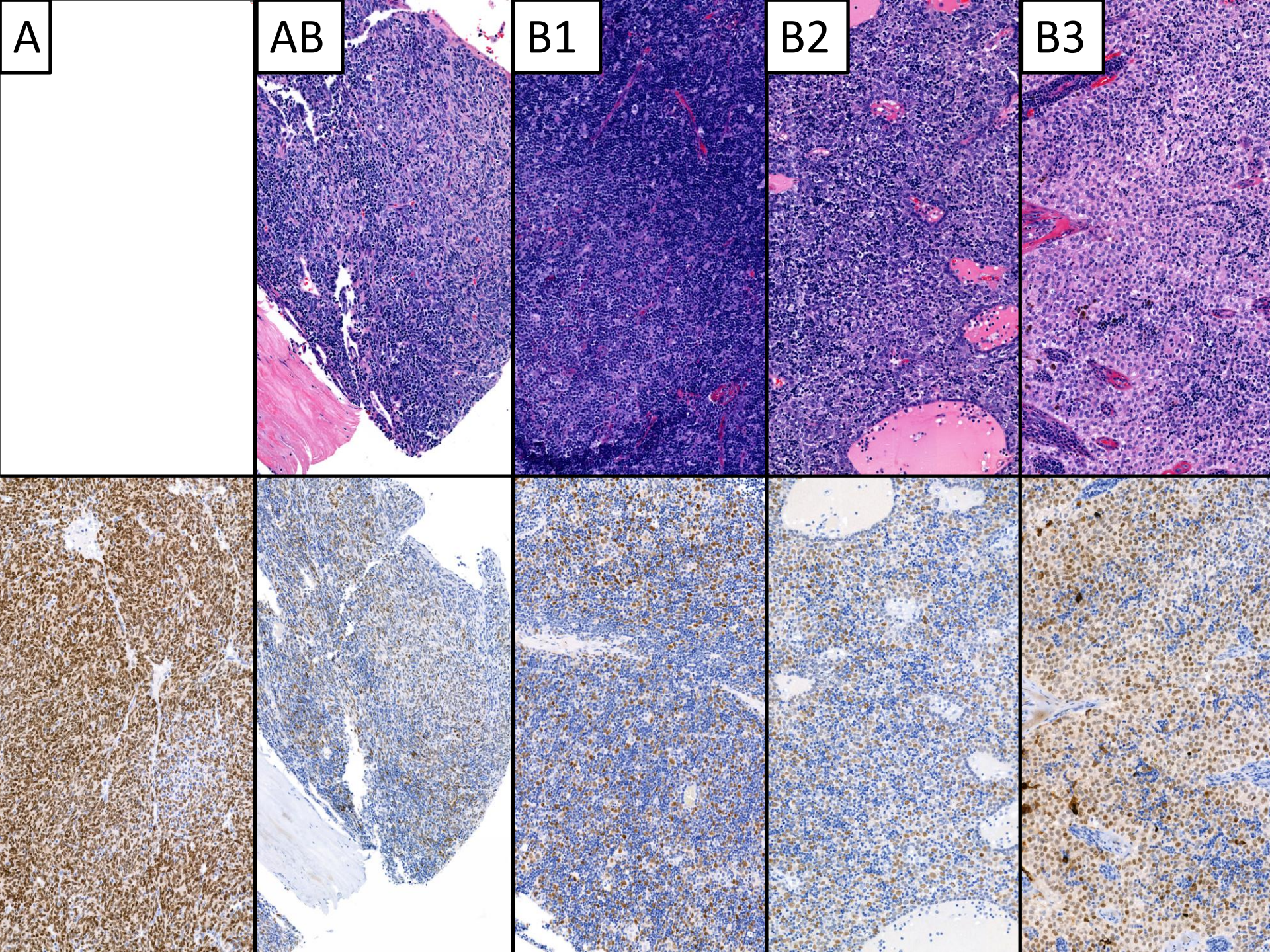
Advanced Search

Search genes by site of expression or sequence

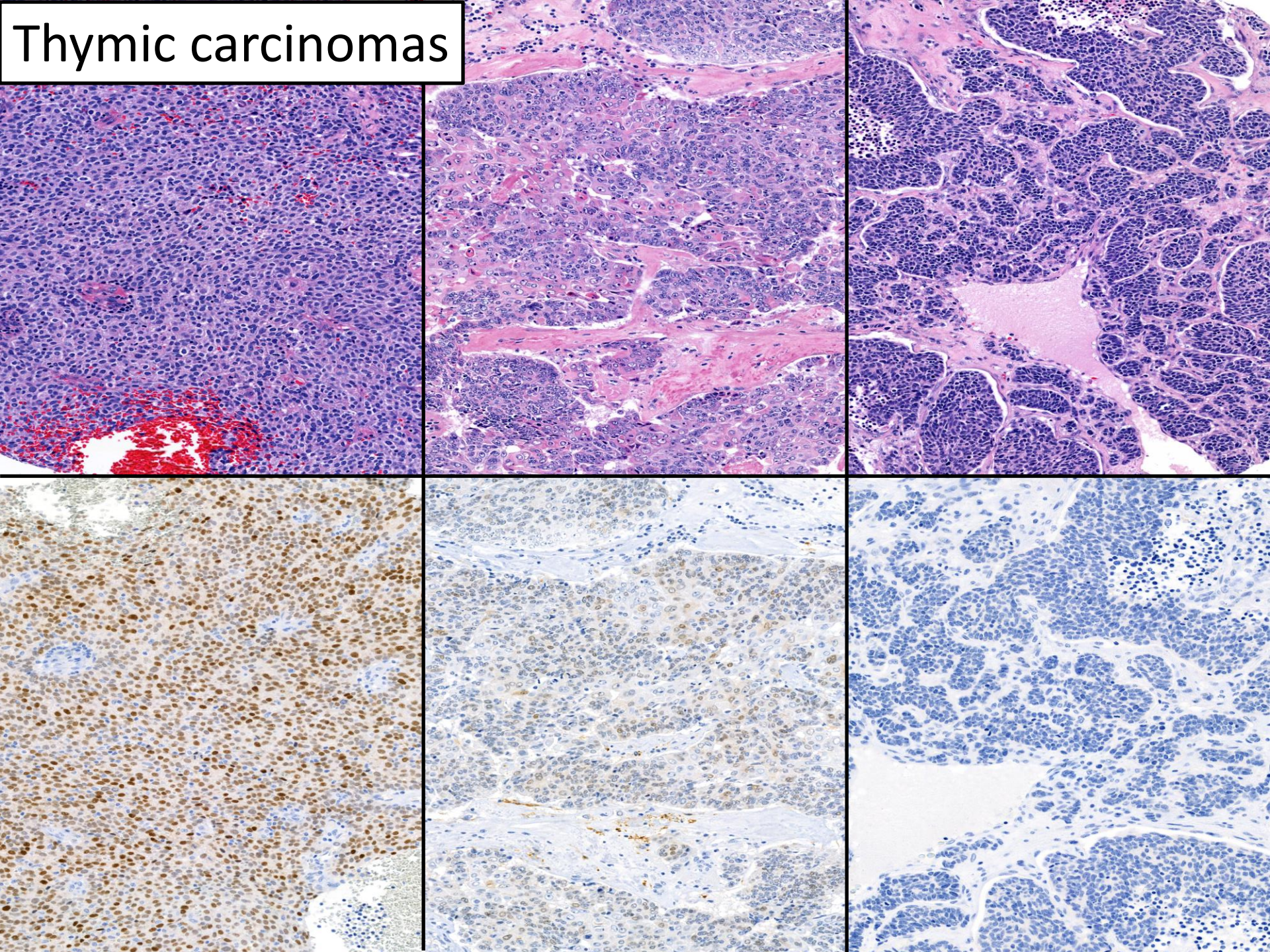
https://gp3.mpg.de/#

PAX1 ISH Expression in E14.5 Mouse




A
AB
B1
B2
B3

Thymic carcinomas



PAX1 Results

Study cohort:
79 thymomas, 10 thymic carcinomas, 175 squamous cell carcinomas (25 lung), 41 urothelial ca, 36 lung adca, 28 RCC, 25 serous, 22 endometroid, 22 PTC, 22 FTC, 10 breast, 5 colon, 5 esophagus, 2 NEC, 1 prostate

PAX1 expression is present in:

91% thymic tumors :

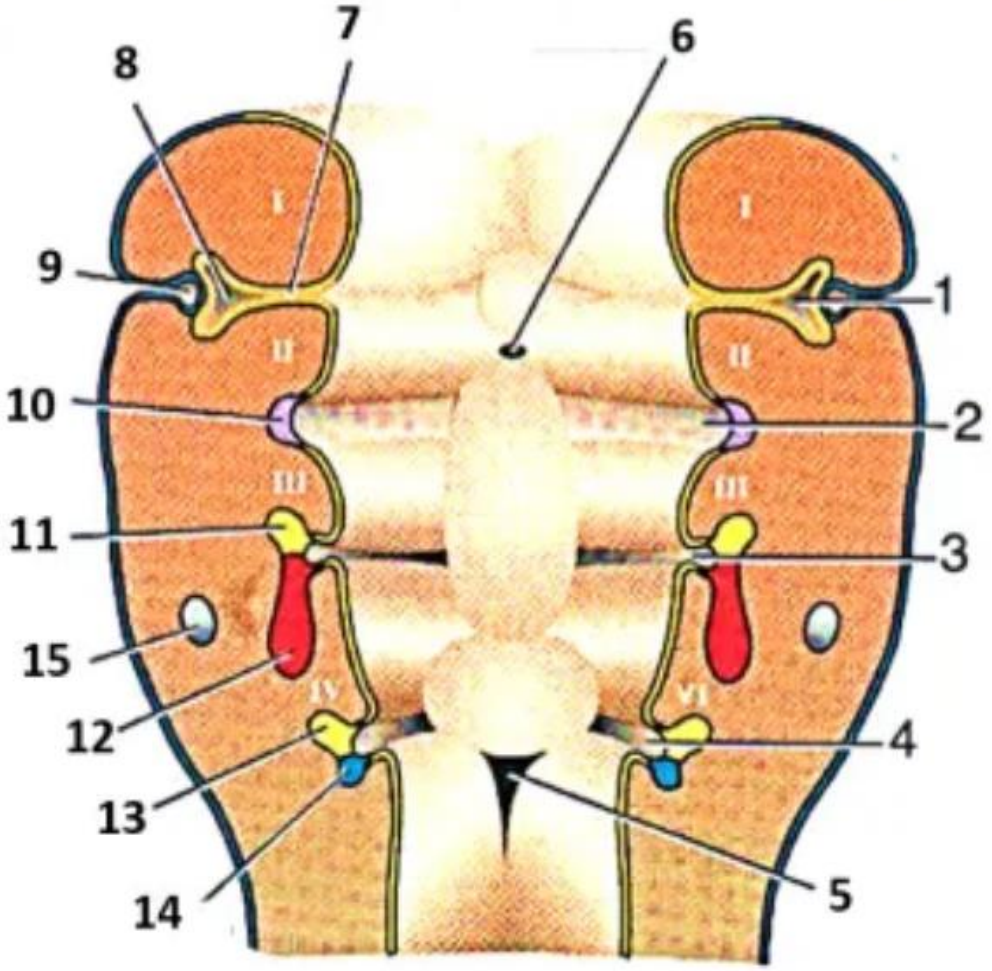
- 94% thymomas (mean/Median H-score-198/210)
- 70% thymic carcinoma (mean/Median H-score- 144/145)

3.5% of non-thymic carcinomas with mean (median) H-score of 15 (5) [p<0.0001%]

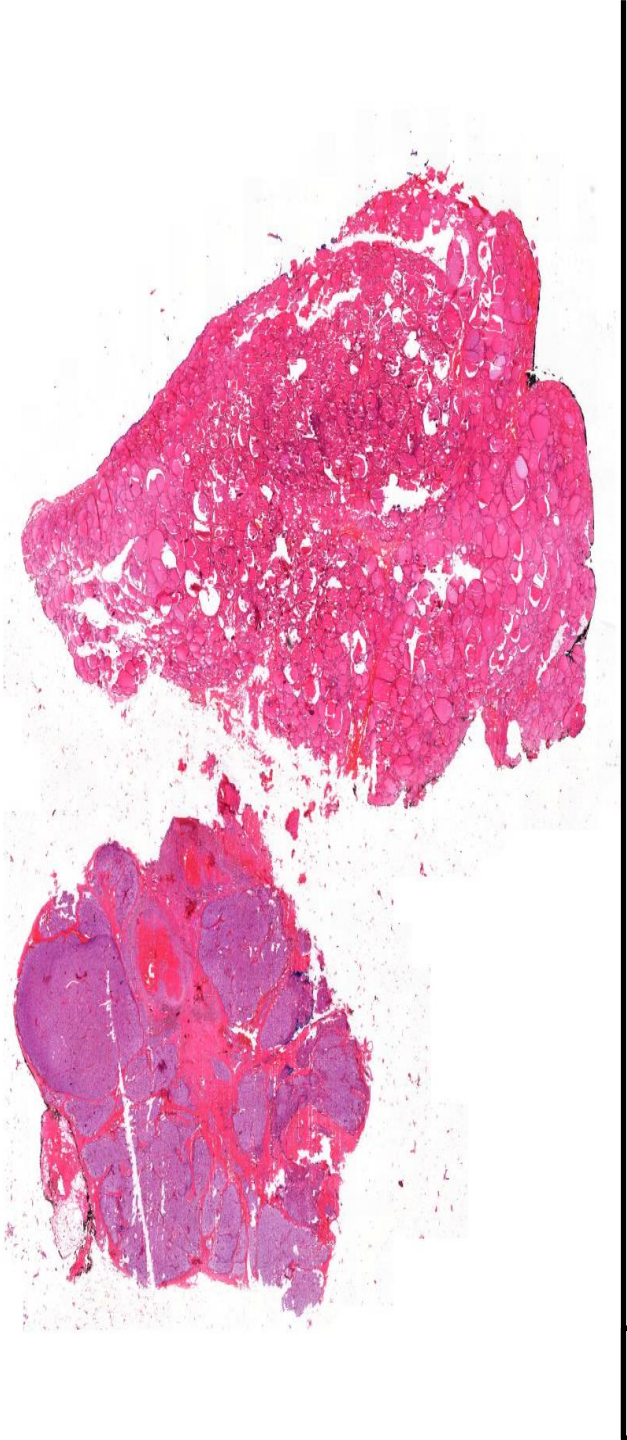
- 6 thyroid carcinomas (14%)
- 4 squamous cell carcinomas (2%[2 lung (8%)])
- 3 urothelial carcinomas (7%)
- 1 breast carcinoma (10%)

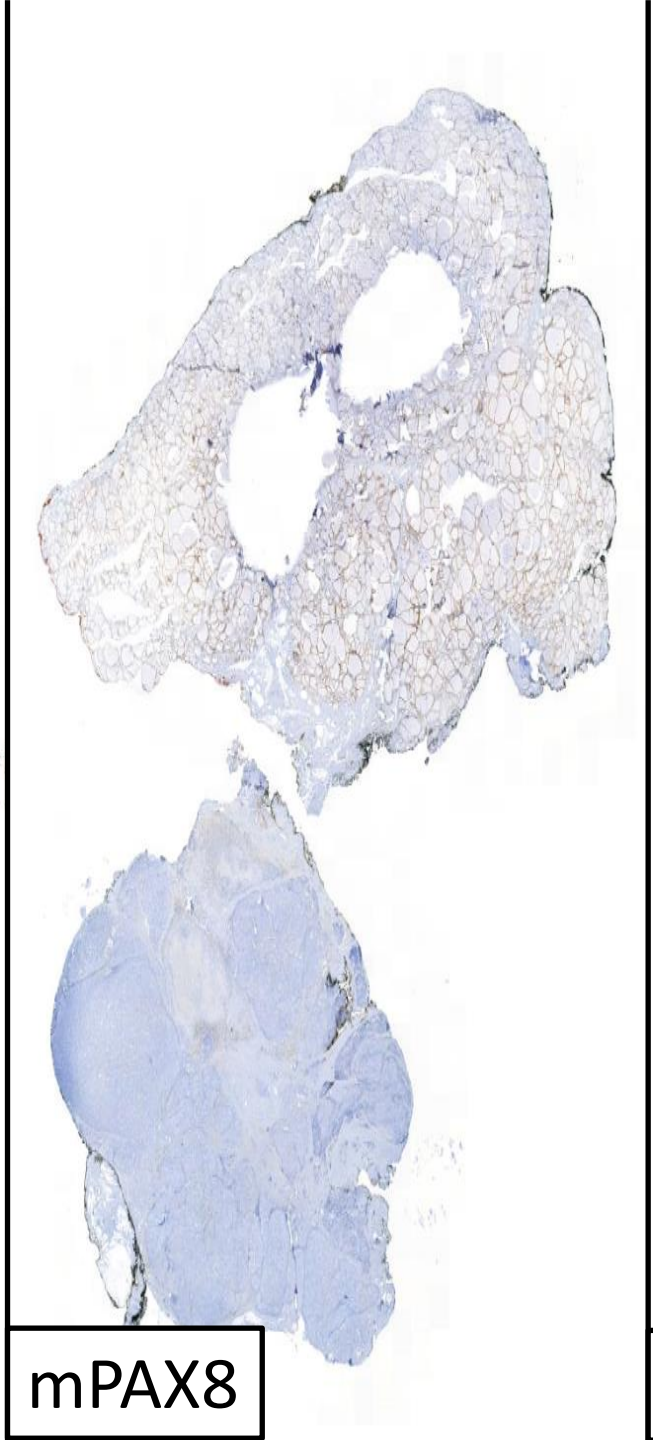
Thymoma Type	(%) Positive	Mean (Median)
A (n=3)	100	221 (200)
AB (n=15)	100	199 (215)
B1 (n=19)	89	190 (220)
B2 (n=22)	95	191 (190)
B3 (n=10)	90	217 (245)

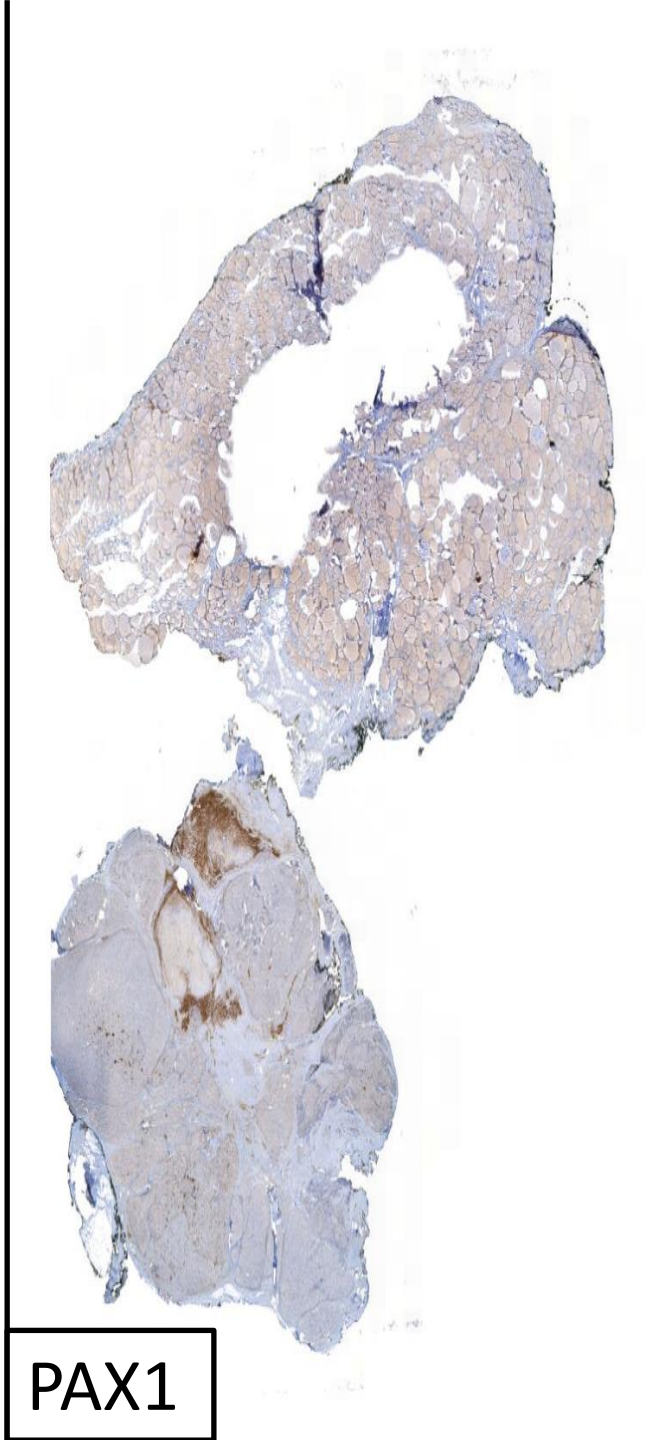
Derivatives of Pharyngeal Pouches

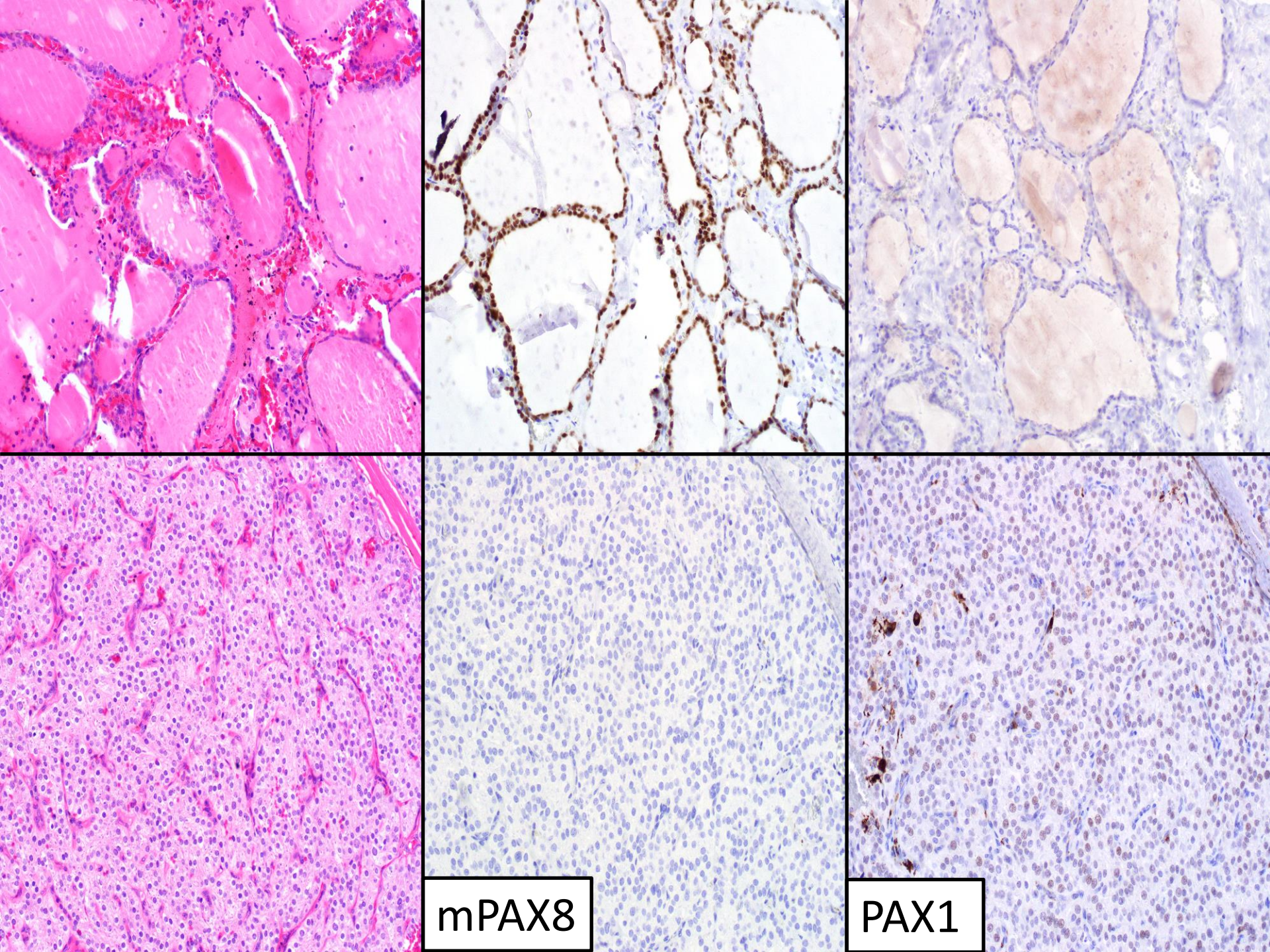


1. 1st pharyngeal pouch
2. 2nd pharyngeal pouch
3. 3rd pharyngeal pouch
4. 4th pharyngeal pouch
5. Laryngeal orifice
6. Foramen cecum
7. Auditory tube
8. Tympanic cavity
9. External auditory meatus
10. Palatine tonsil
11. Inferior parathyroid gland
12. Thymus
13. Superior parathyroid gland
14. Ultimobranchial body
15. Cervical sinus
16. 1st pharyngeal cleft




mPAX8


PAX1


mPAX8
PAX1

Andrew Bellizzi
@IHC_guy

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Kudos to Dr. Charville's Team!!!

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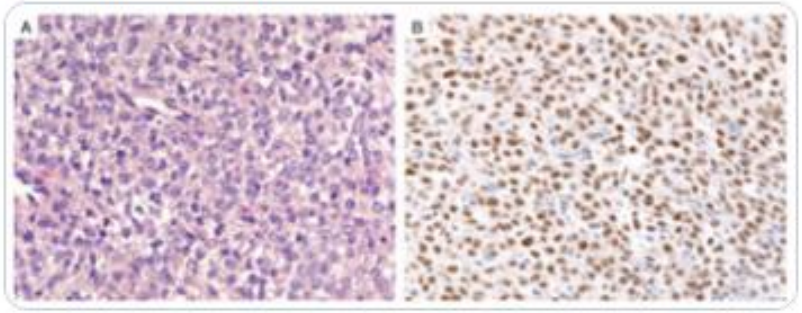


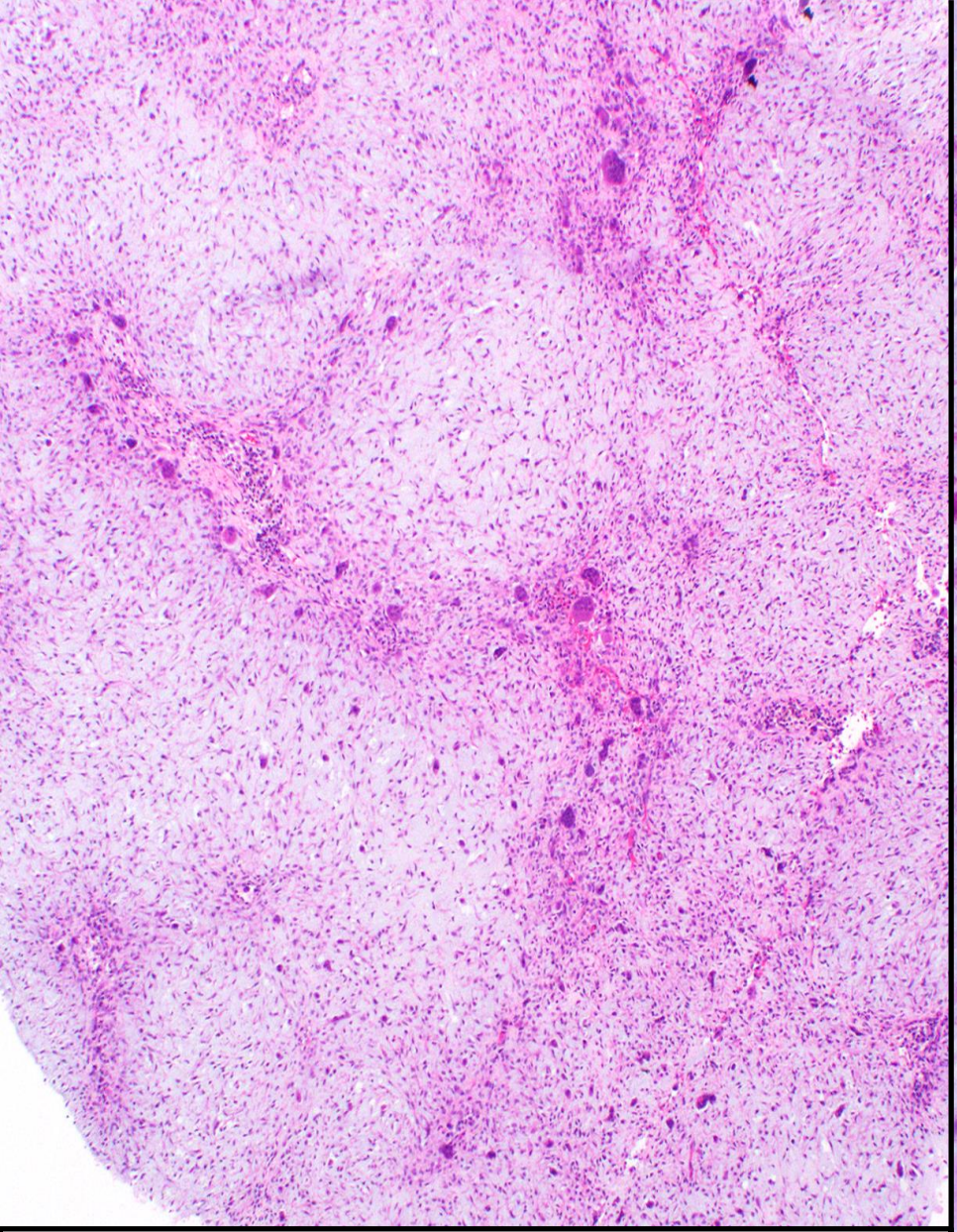
5:14 PM · Aug 20, 2020 · Twitter Web App

The PathPod Podcast
@PathPod

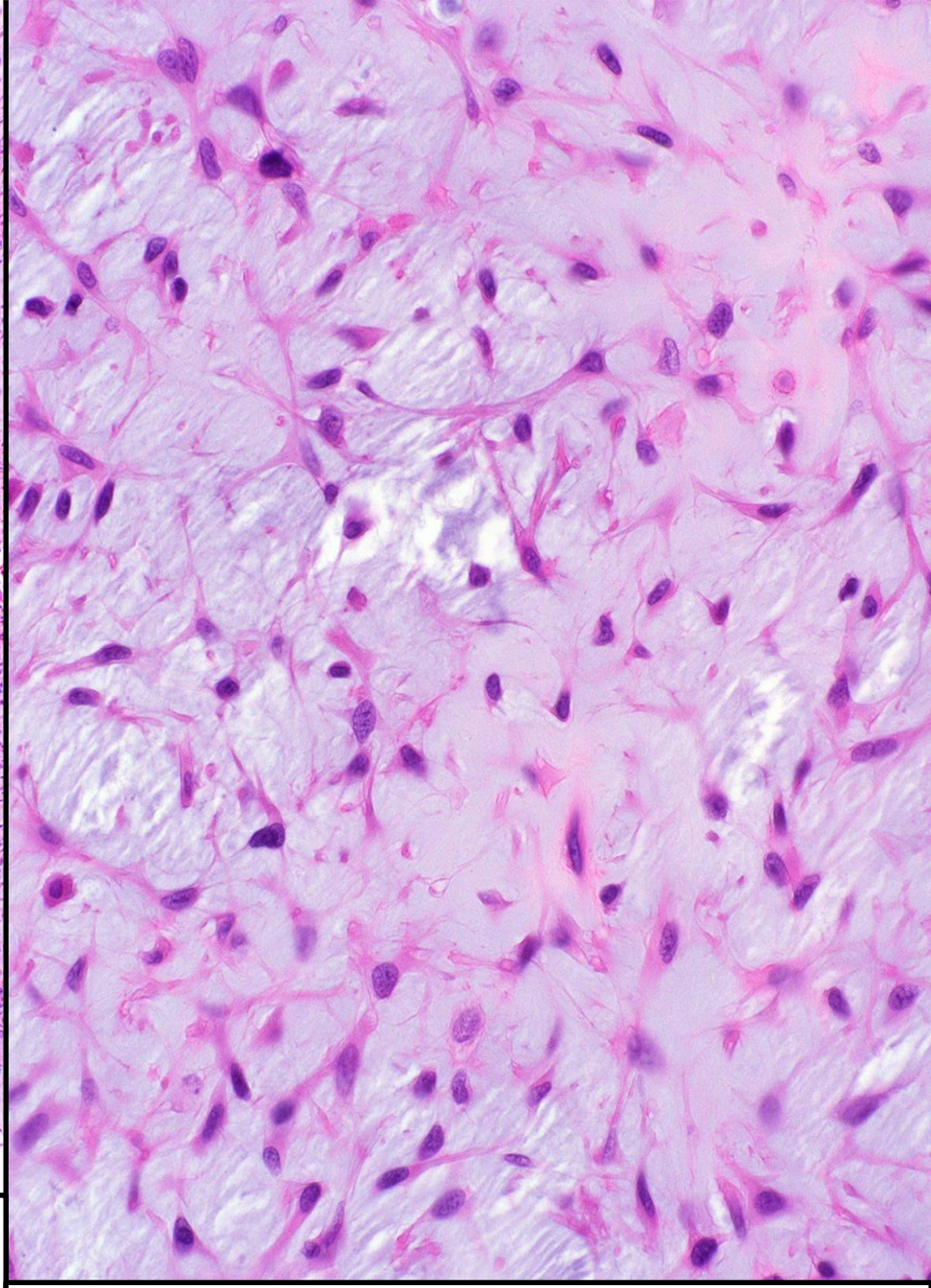
The #PathPod crew is taking a break this week to enjoy #ASCP2020, but look what the chromogen sibling have cooking for next week! Welcome @Greg_Charville to #IHCtalk on DDIT3 IHC (journals.lww.com/ajsp/Abstract/...) and welcome to Twitter!

@sanamloghavi @IHC_guy @MArnold_PedPath

5:34 PM · Sep 10, 2020 · Twitter for iPhone



Lobulated, more cellular periphery, osteoclast-like GCs



Bi- & multipolar stellate cells

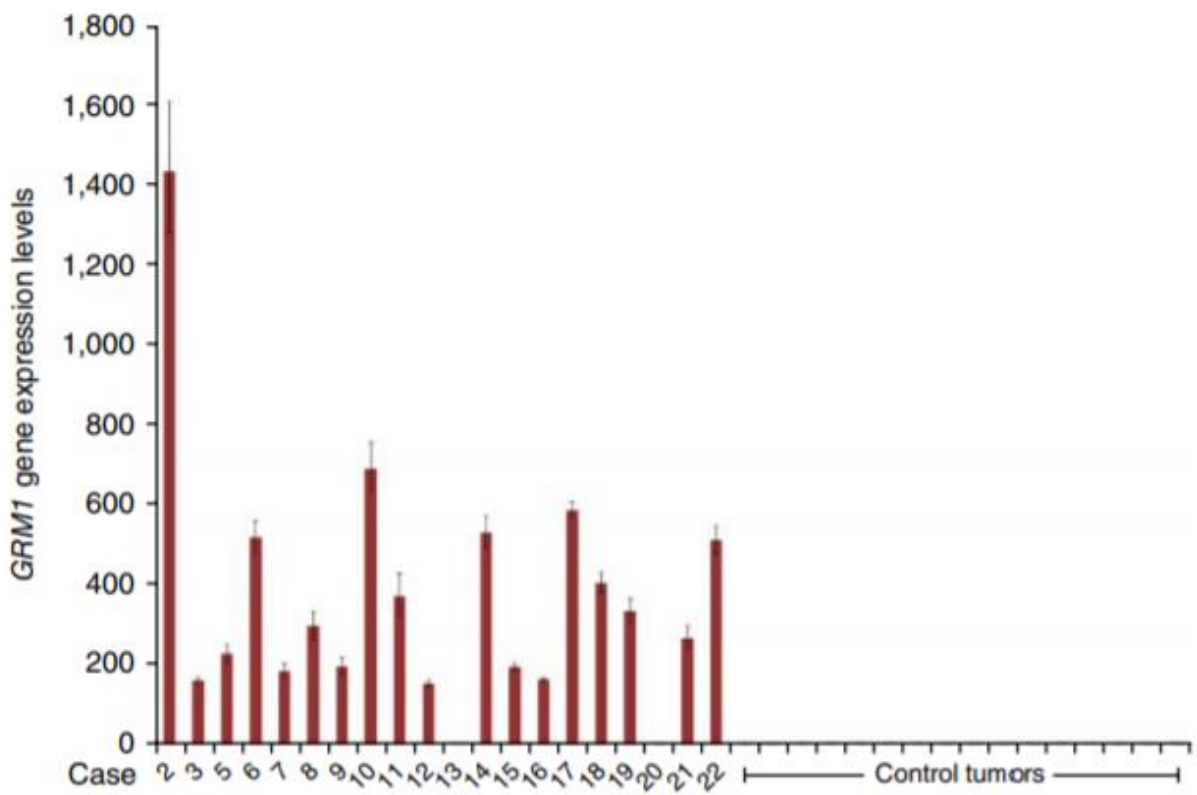
The Straw That Broke the Camel's Back

Preliminary Diagnosis**

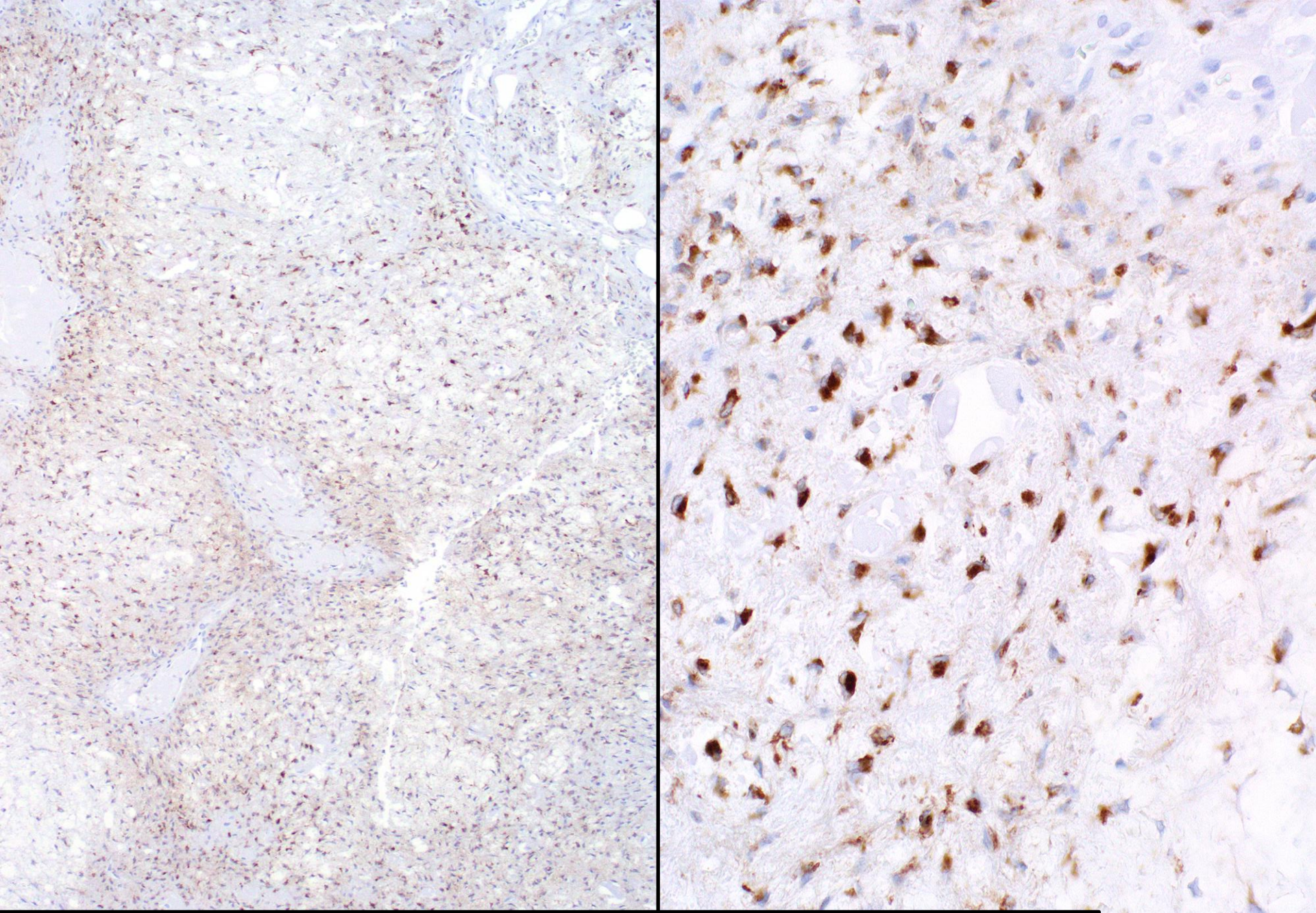
A. Bone, Specify: **Looks like a hypercellular cartilaginous proliferation, I think there is some osteoid production although the decal makes it hard, favor chondroblastic osteosarcoma but I guess it could be something benign**

ND to JJD

Genetics of Chondromyxoid Fibroma



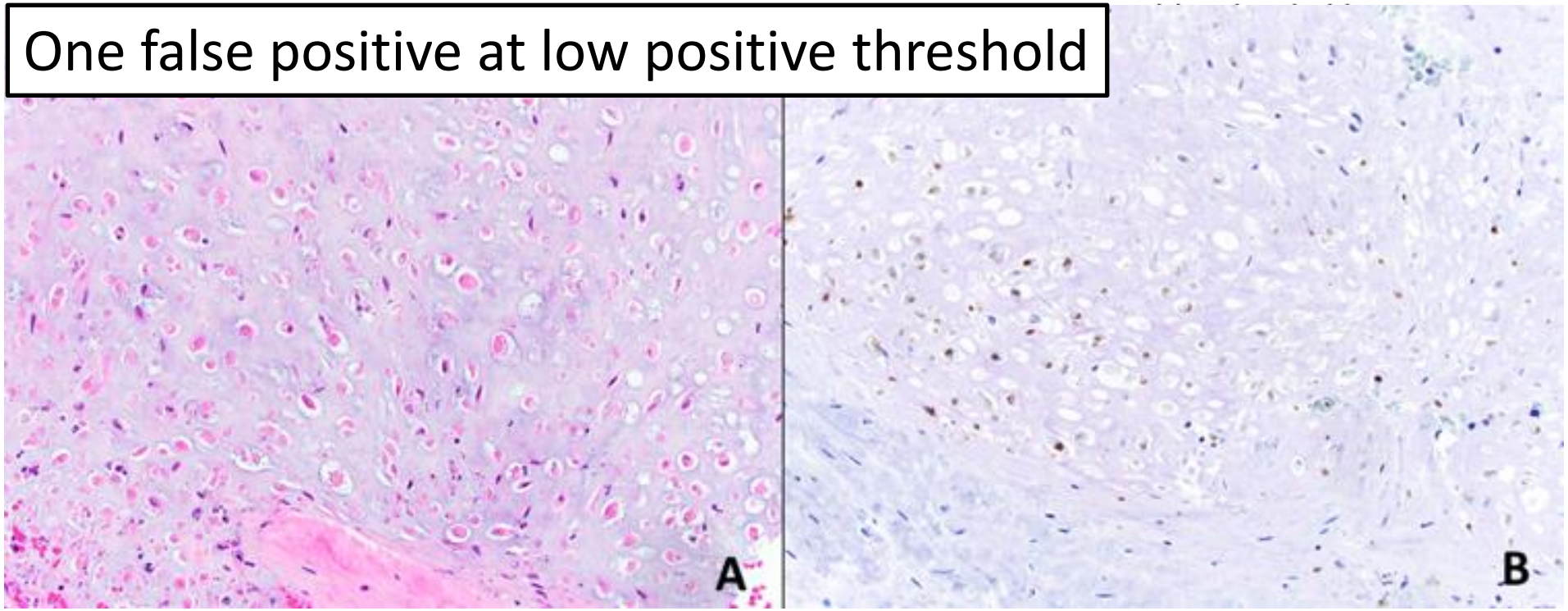
18/20 (90%) CMF showed 100-1400 fold increased expression



mGluR1 (clone JM11-61; Novus; LpH; 1:200; 30’-30’

Test Characteristics

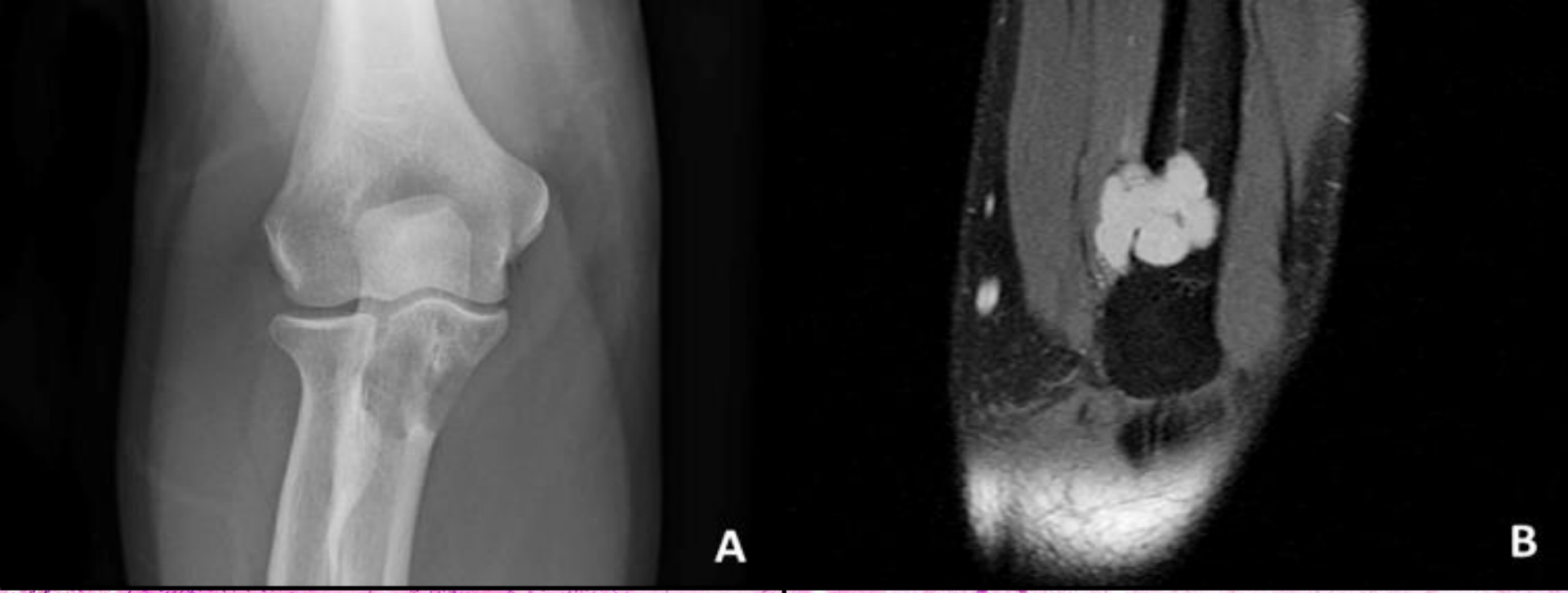
One false positive at low positive threshold

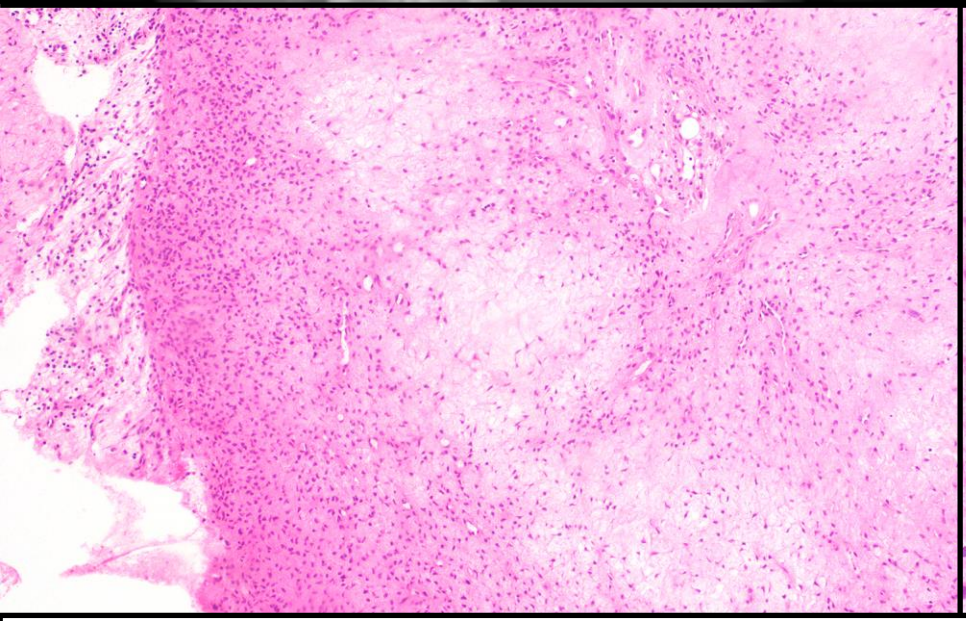


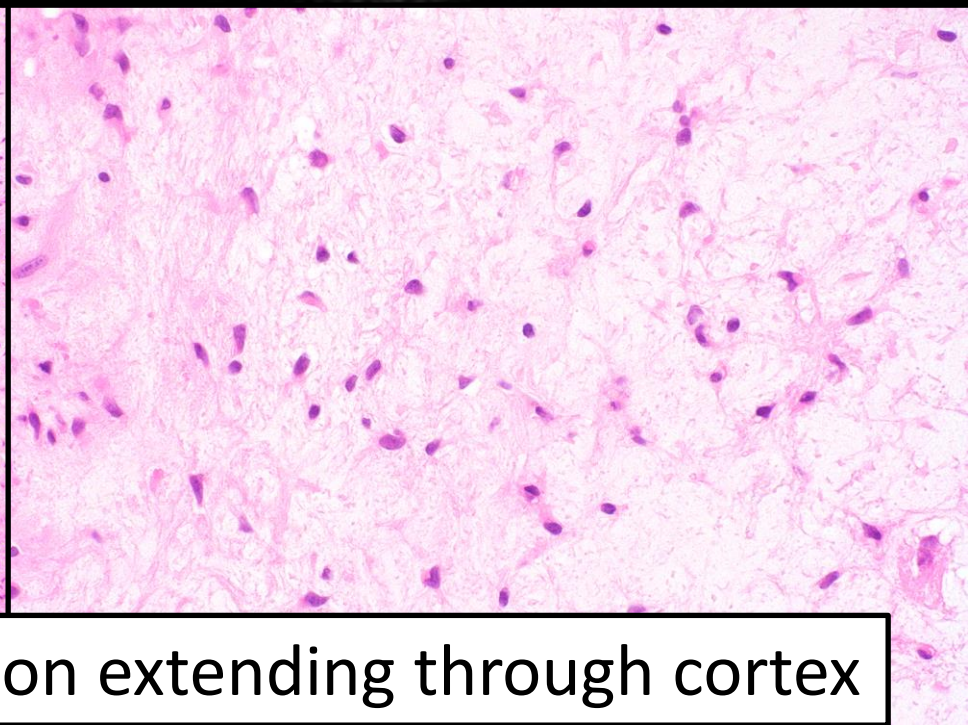
Diagnostic mimics: chondroblastoma (20), giant cell tumor of bone (12), enchondroma (9), myxoma of none (1), ACT/G1 CS, G2 CS (9), HG/DD CS (3), chondroblastic OS (7), chordoma (4)

Diagnostic Utility of mGluR1

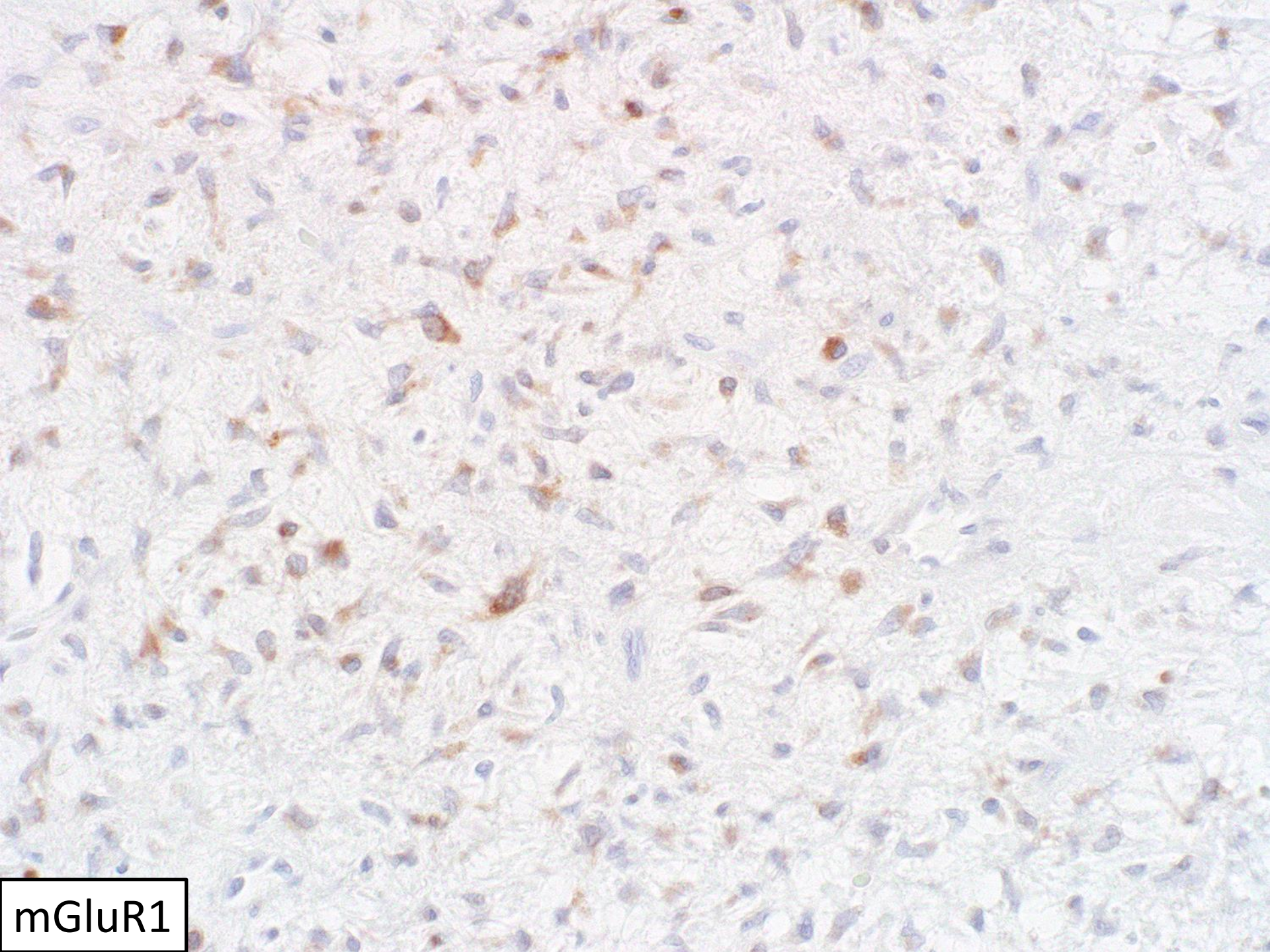
1. Small core biopsies/limited sampling
2. Aggressive radiographic features (e.g., extension into surrounding soft tissue)
3. Atypical histologic appearance (18% with bizarre cytologic features in Mayo Clinic series n=278)
4. Unusual location
5. Limited pathologist experience

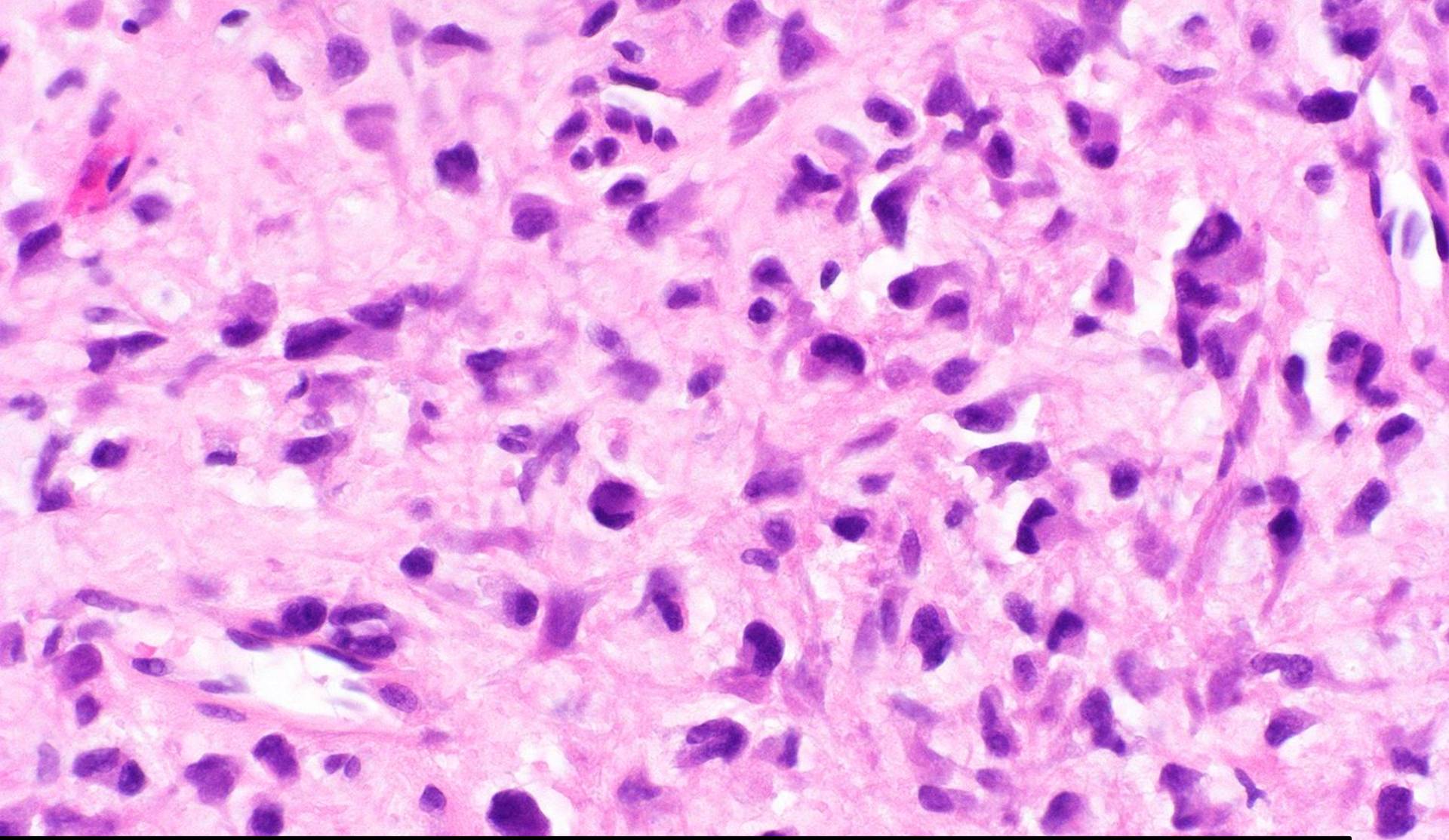




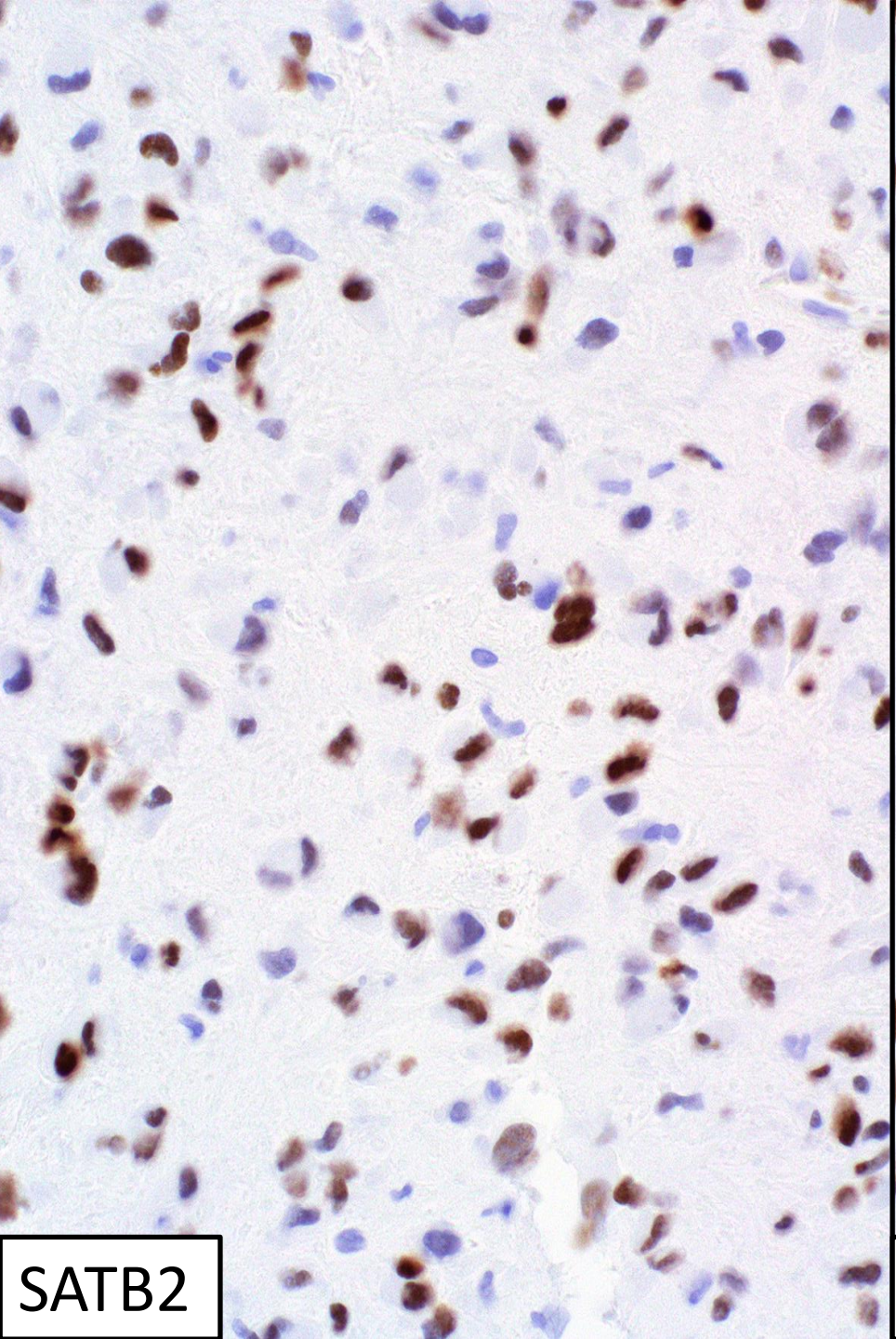


49-year-old with lytic ulnar lesion extending through cortex


mGluR1



25W with 2.3 cm mass in the distal fibula with extension to the medial cortex and associated periosteal reaction and soft tissue edema



SATB2

ERG expression in chondrogenic bone and soft tissue tumours

Wonwoo Shon,[1] Andrew L Folpe,[2] Karen J Fritchie[2]

[1]Department of Pathology, Immunology, and Laboratory Medicine, University of Florida College of Medicine, Gainesville, Florida, USA
[2]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA

Correspondence to
Dr Karen J Fritchie, Department of Laboratory Medicine and Pathology, 200 First Street SW, Mayo Clinic, Rochester, MN 55905, USA; fritchie.karen@mayo.edu

Received 18 August 2014
Revised 7 October 2014
Accepted 19 October 2014
Published Online First 5 November 2014

ABSTRACT

Aim We studied ERG expression in a large series of chondrogenic bone and soft tissue tumours to assess the value of ERG as a possible marker of chondroid/cartilaginous differentiation.

Methods Formalin-fixed, paraffin-embedded whole sections from 111 bone and soft tissue tumours with chondroid differentiation or a morphology that may mimic cartilaginous differentiation were retrieved. Immunohistochemistry was performed using anti-ERG monoclonal antibody directed against the N terminus. Nuclear staining was scored as negative (<5%), 1+ (5%–25%), 2+ (26%–50%), 3+ (>51%).

Results Nuclear ERG expression was seen in all cases of soft tissue chondroma (8), chondromyxoid fibroma (7), chondroblastic osteosarcoma (6) and clear cell chondrosarcoma (1). 10/12 conventional chondrosarcomas were also positive for ERG. In cases of dedifferentiated chondrosarcoma, the well-differentiated component was positive in 7/9 cases, while all dedifferentiated foci were negative. In cases of mesenchymal chondrosarcoma, the hyaline cartilage component was positive in 2/4 cases, whereas the primitive component in all cases was negative. Variable positivity was identified in extraskeletal myxoid chondrosarcomas (4/9), chondroblastomas (3/8) and mixed tumours/myoepitheliomas (2/11). Only 1/12 chordoma was positive for ERG (1+). Interestingly, 15/17 enchondromas were negative for ERG.

Conclusions In this study, we further characterise the expression of ERG in mesenchymal tumours and found relatively constant nuclear ERG expression in selected chondrogenic tumours including conventional chondrosarcoma, chondromyxoid fibroma, chondroblastic osteosarcoma and clear cell chondrosarcoma. We also show that ERG may be a helpful ancillary tool in certain select diagnostic scenarios and that awareness of ERG expression in tumours with cartilaginous differentiation is important.

INTRODUCTION

ERG is a member of the ETS family of regulatory transcription factors with diverse biological roles that include the regulation of angiogenesis and cell apoptosis of endothelial cells.[1] Consequently, expression of ERG has been shown to be a constant feature of tumours with endothelial differentiation.[2 3] Interestingly, several experimental studies have demonstrated that *ERG* is a regulatory gene of cartilage skeletogenesis and may have a crucial role in permanent cartilage tissue development including maintenance of chondrocytes in a differentiated state.[4 5] Moreover, its expression in precartilage primordium and cartilaginous components of various organ systems in developing and adult mouse tissues has been confirmed.[6] Prompted by the identification of ERG immunoreactivity in several soft tissue chondromas in our consultation practice, we undertook a large retrospective study of ERG expression in a broad variety of chondrogenic mesenchymal tumours to assess its diagnostic use as a marker of chondrogenic/cartilaginous differentiation, given its role as a transcription factor in the regulation.

MATERIALS AND METHODS

This study was approved by the Mayo Clinic Institutional Review Board. Formalin-fixed, paraffin-embedded whole tissue sections from 111 chondrogenic bone and soft tissue tumours were obtained from the archives of the Mayo Clinic including enchondroma (17 cases), dedifferentiated chondrosarcoma (14 cases), conventional chondrosarcoma (12 cases), chordoma (12 cases), mixed tumour/myoepithelioma (11 cases), extraskeletal myxoid chondrosarcoma (9 cases), chondroblastoma (8 cases), soft tissue chondromas (8 cases), chondromyxoid fibroma (7 cases), chondroblastic osteosarcoma (6 cases), mesenchymal chondrosarcoma (4 cases), chondroblastoma-like chondrosarcoma (2 cases) and clear cell chondrosarcoma (1 case). The histological diagnoses were confirmed. A representative tissue block from each case was selected and immunostained for anti-ERG monoclonal antibody to the N terminus (9FY; 1:50–1:100, BioCare) using heat-induced epitope retrieval. The nuclear immunoreactivity of the tumour cells was semiquantitatively assessed using the following scoring scheme: negative (<5% of cells positive), 1+ (5%–25% of cells positive), 2+ (26%–50% of cells positive) and 3+ (>51% of cells positive). When available, normal endothelial cells invariably served as a positive internal control and cases without positive internal controls (most likely due to harsh decalcification) were not included in the study.

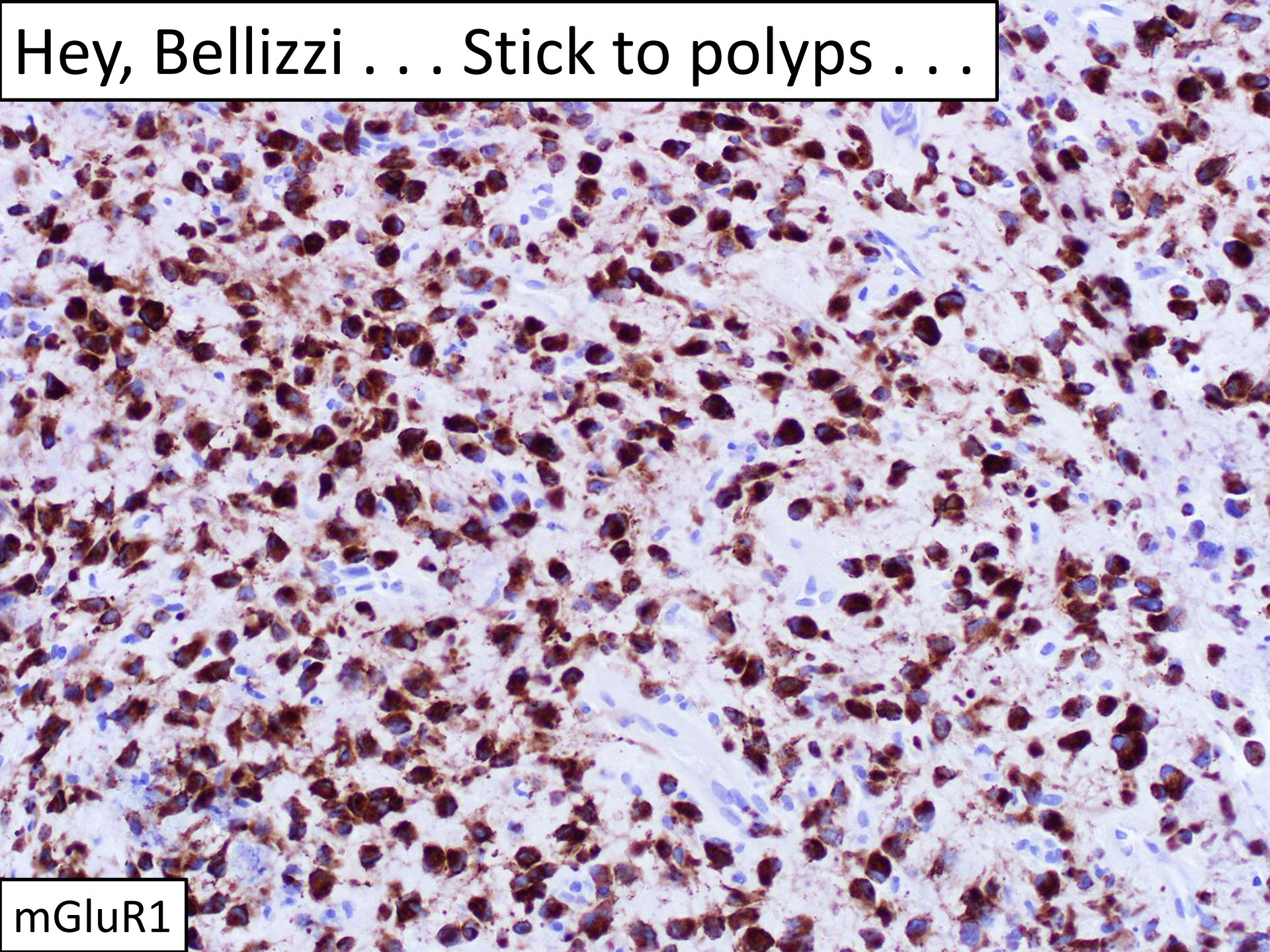
RESULTS

The immunohistochemical results are summarised in table 1. All cases of soft tissue chondroma (8/8), chondromyxoid fibroma (7/7), chondroblastic osteosarcoma (6/6) and clear cell chondrosarcoma (1/1) were positive for ERG (figure 1A–F). Nuclear expression of ERG was also observed in the majority of conventional chondrosarcomas (10/12; 83%; figure 1G, H). In all bone tumours, both osteoblasts and osteocytes are consistently negative for ERG. When ERG expression was evaluated separately in the components of dedifferentiated chondrosarcoma, the well-differentiated cartilaginous component was positive in seven of nine cases (78%), while all dedifferentiated foci were negative (figure 2A, B). In mesenchymal chondrosarcomas the hyaline cartilage component was positive in 50% of cases (2/4), whereas the primitive round

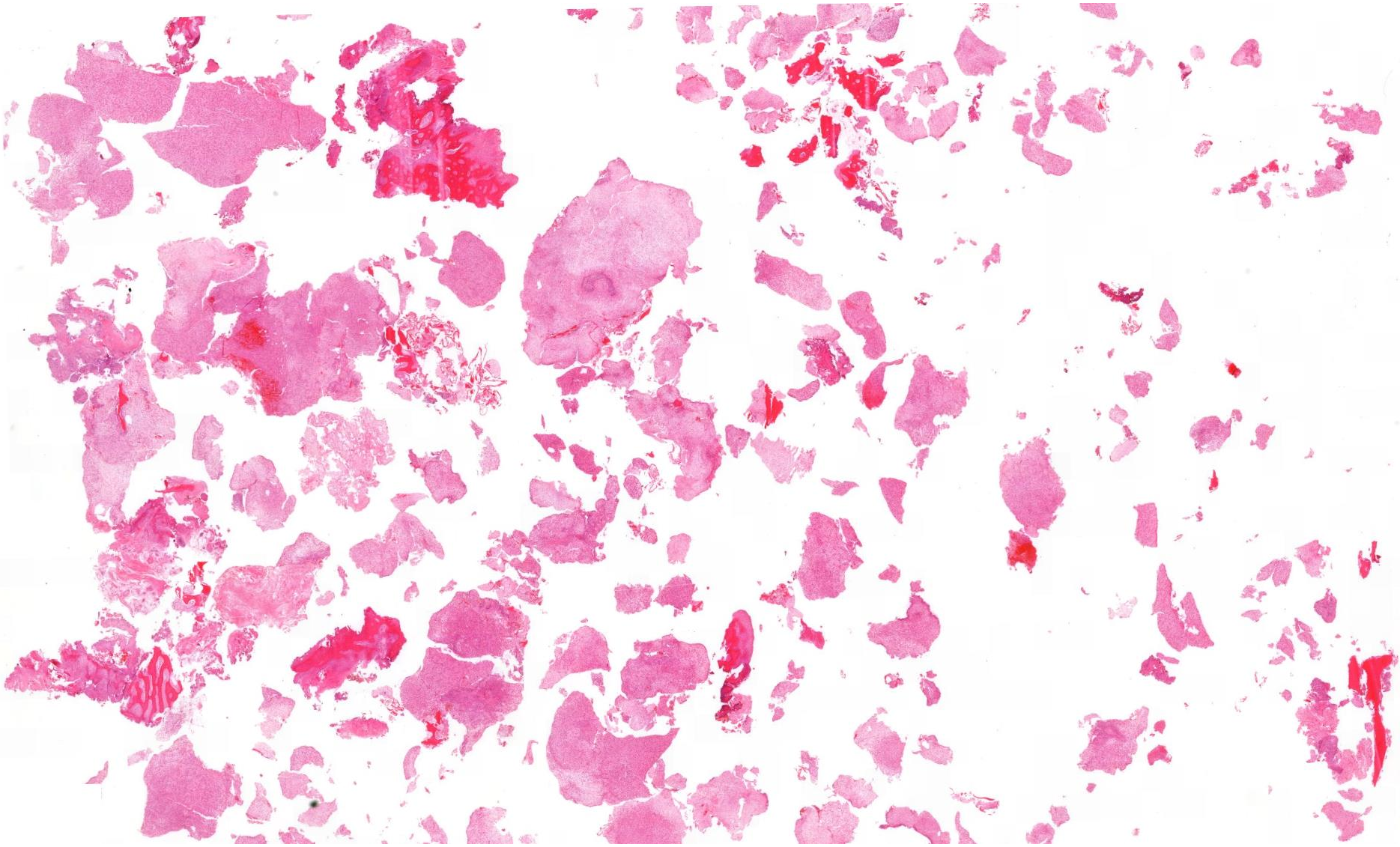
To cite: Shon W, Folpe AL, Fritchie KJ. *J Clin Pathol* 2015;**68**:125–129.

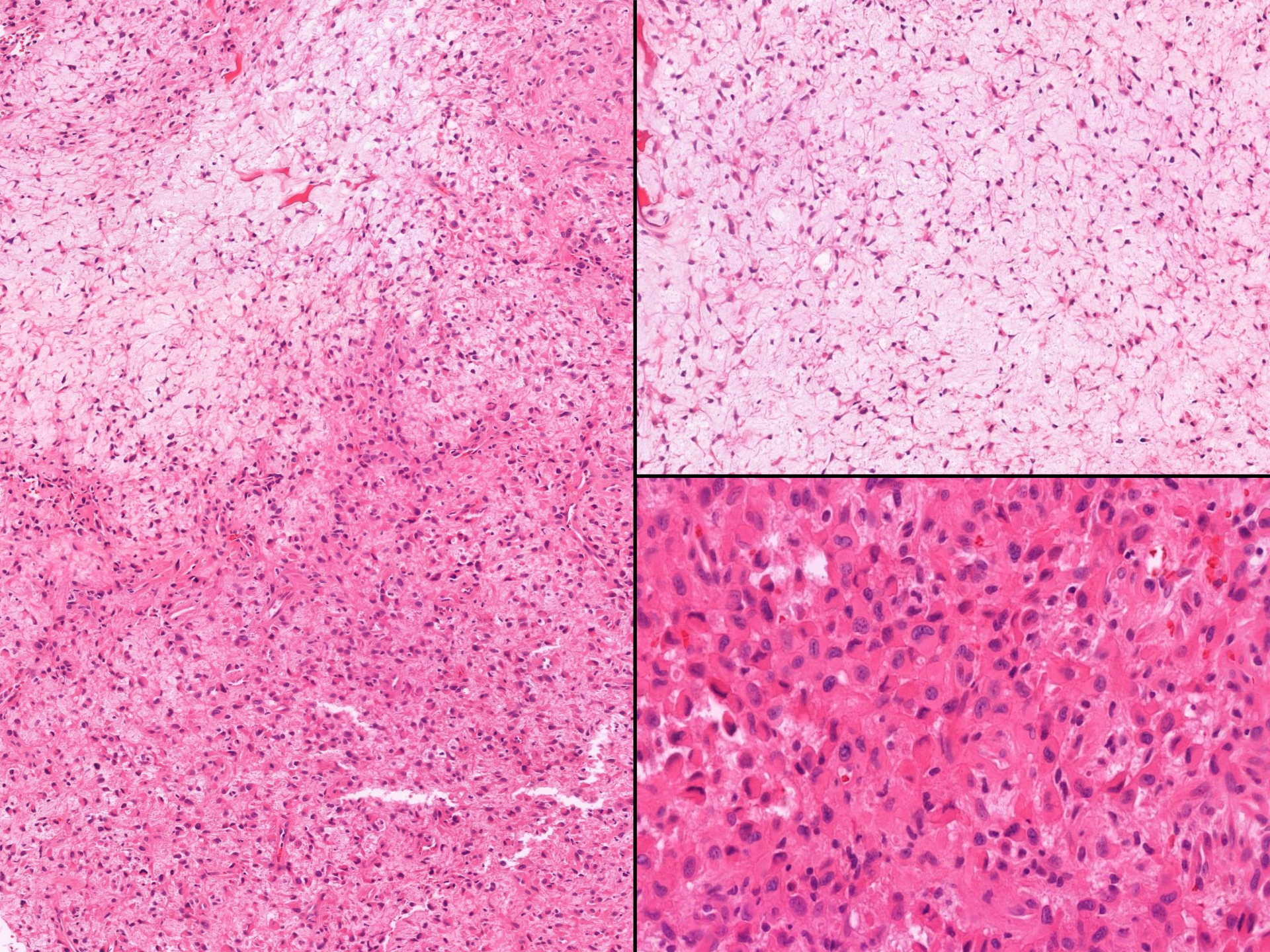
ERG

Hey, Bellizzi . . . Stick to polyps . . .



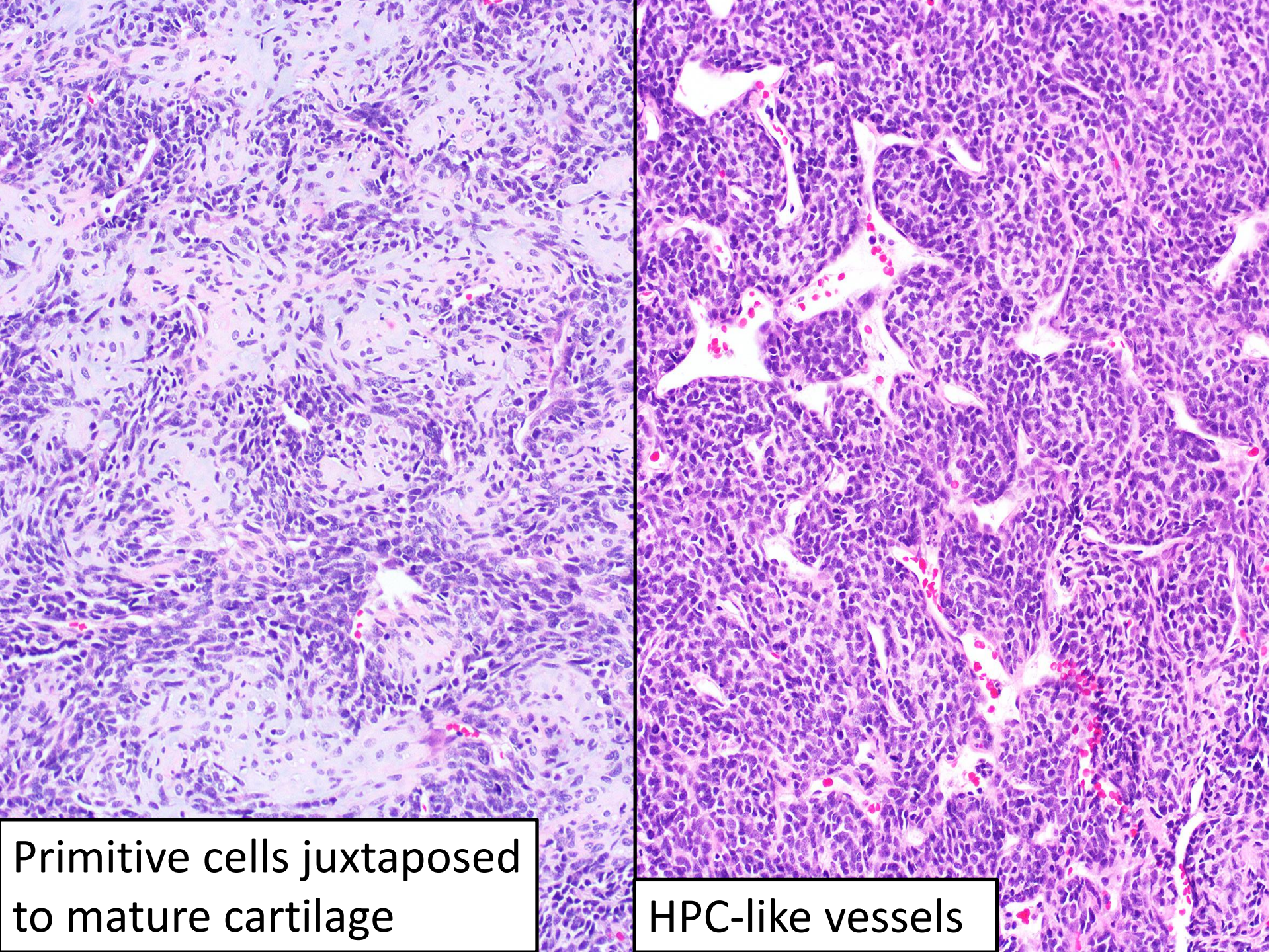
mGluR1

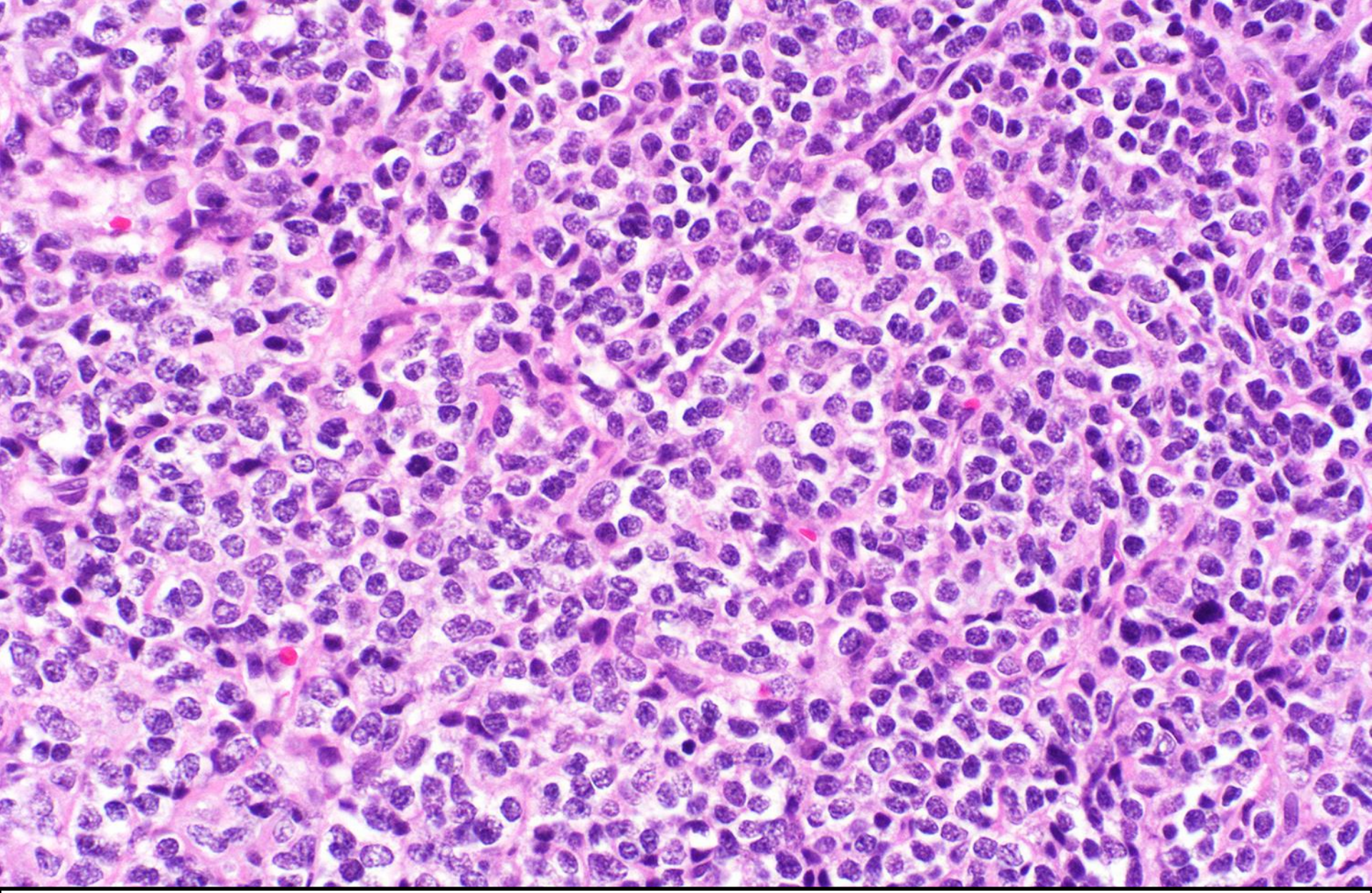




Hey, what antibody do you run for NKX3.1?

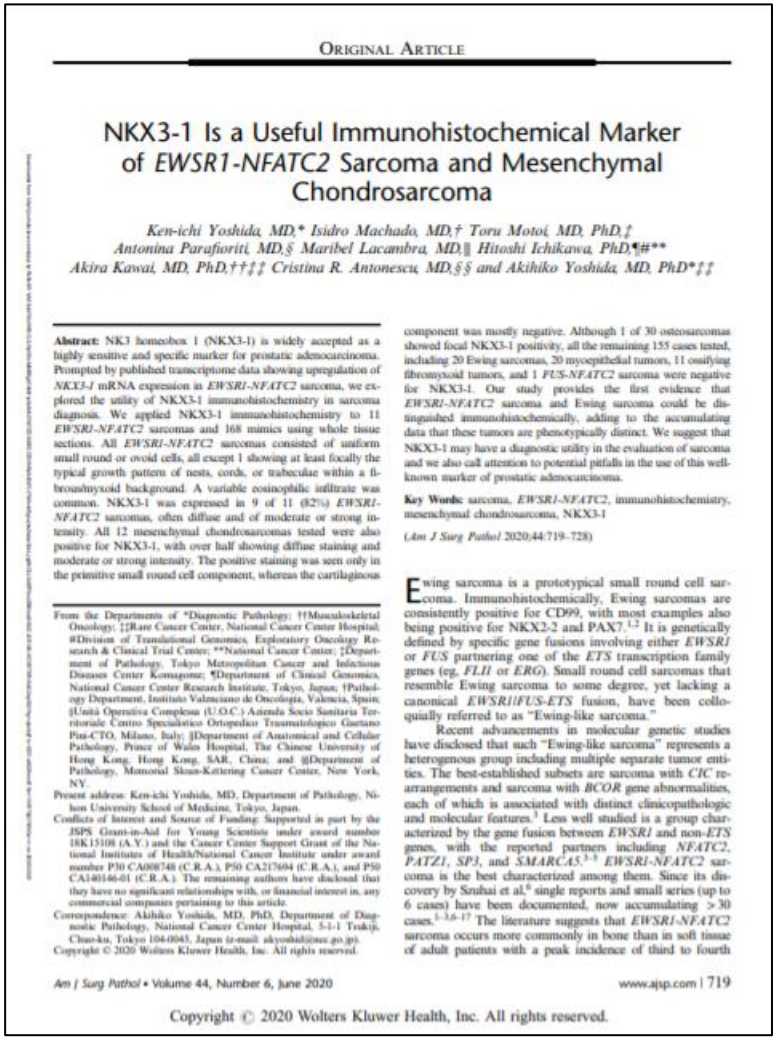
--Jason L Hornick, MD, PhD


Primitive cells juxtaposed to mature cartilage
HPC-like vessels



Undifferentiated round cells; no mature cartilage; no HPC-like vessels; these 3 images all from same case; tumor freq. CD99+

NKX3.1 Expression in Mesenchymal Chondrosarcoma



ORIGINAL ARTICLE

NKX3-1 Is a Useful Immunohistochemical Marker of *EWSR1-NFATC2* Sarcoma and Mesenchymal Chondrosarcoma

Ken-ichi Yoshida, MD, Isidro Machado, MD,† Toru Motoi, MD, PhD,‡ Antonina Parafioriti, MD,§ Maribel Lacambra, MD,‖ Hitoshi Ichikawa, PhD,¶#** Akira Kawai, MD, PhD,††‡‡ Cristina R. Antonescu, MD,§§ and Akihiko Yoshida, MD, PhD*‡‡*

Abstract: NK3 homeobox 1 (NKX3-1) is widely accepted as a highly sensitive and specific marker for prostatic adenocarcinoma. Prompted by published transcriptome data showing upregulation of *NKX3-1* mRNA expression in *EWSR1-NFATC2* sarcoma, we explored the utility of NKX3-1 immunohistochemistry in sarcoma diagnosis. We applied NKX3-1 immunohistochemistry to 11 *EWSR1-NFATC2* sarcomas and 168 mimics using whole tissue sections. All *EWSR1-NFATC2* sarcomas consisted of uniform small round or ovoid cells, all except 1 showing at least focally the typical growth pattern of nests, cords, or trabeculae within a fibrous/myxoid background. A variable eosinophilic infiltrate was common. NKX3-1 was expressed in 9 of 11 (82%) *EWSR1-NFATC2* sarcomas, often diffuse and of moderate or strong intensity. All 12 mesenchymal chondrosarcomas tested were also positive for NKX3-1, with over half showing diffuse staining and moderate or strong intensity. The positive staining was seen only in the primitive small round cell component, whereas the cartilaginous component was mostly negative. Although 1 of 30 osteosarcomas showed focal NKX3-1 positivity, all the remaining 155 cases tested, including 20 Ewing sarcomas, 20 myoepithelial tumors, 11 ossifying fibromyxoid tumors, and 1 *FUS-NFATC2* sarcoma were negative for NKX3-1. Our study provides the first evidence that *EWSR1-NFATC2* sarcoma and Ewing sarcoma could be distinguished immunohistochemically, adding to the accumulating data that these tumors are phenotypically distinct. We suggest that NKX3-1 may have a diagnostic utility in the evaluation of sarcoma and we also call attention to potential pitfalls in the use of this well-known marker of prostatic adenocarcinoma.

Key Words: sarcoma, *EWSR1-NFATC2*, immunohistochemistry, mesenchymal chondrosarcoma, NKX3-1

(*Am J Surg Pathol* 2020;44:719–728)

Ewing sarcoma is a prototypical small round cell sarcoma. Immunohistochemically, Ewing sarcomas are consistently positive for CD99, with most examples also being positive for NKX2-2 and PAX7.[1,2] It is genetically defined by specific gene fusions involving either *EWSR1* or *FUS* partnering one of the *ETS* transcription family genes (eg, *FLI1* or *ERG*). Small round cell sarcomas that resemble Ewing sarcoma to some degree, yet lacking a canonical *EWSR1*/*FUS-ETS* fusion, have been colloquially referred to as "Ewing-like sarcoma."

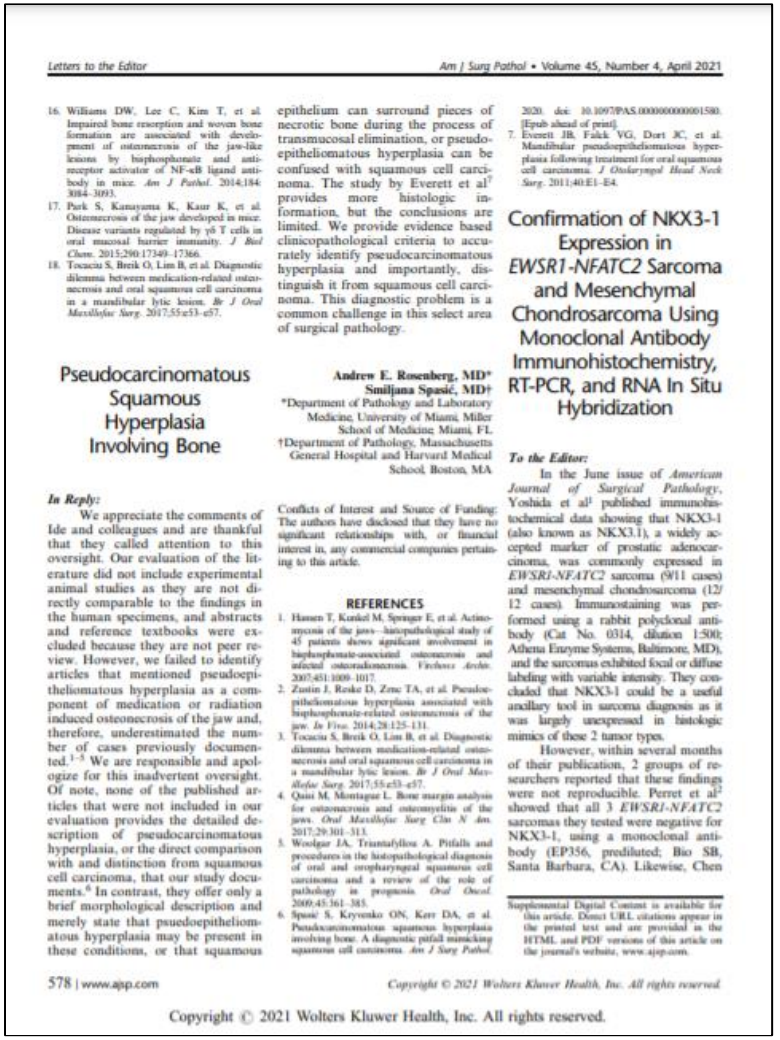
Recent advancements in molecular genetic studies have disclosed that such "Ewing-like sarcoma" represents a heterogenous group including multiple separate tumor entities. The best-established subsets are sarcoma with *CIC* rearrangements and sarcoma with *BCOR* gene abnormalities, each of which is associated with distinct clinicopathologic and molecular features.[1] Less well studied is a group characterized by the gene fusion between *EWSR1* and non-*ETS* genes, with the reported partners including *NFATC2*, *PATZ1*, *SP3*, and *SMARCA5*.[3–5] *EWSR1-NFATC2* sarcoma is the best characterized among them. Since its discovery by Szuhai et al,[6] single reports and small series (up to 6 cases) have been documented, now accumulating >30 cases.[1–3,6–17] The literature suggests that *EWSR1-NFATC2* sarcoma occurs more commonly in bone than in soft tissue of adult patients with a peak incidence of third to fourth

From the Departments of *Diagnostic Pathology; ††Musculoskeletal Oncology; ‡‡Rare Cancer Center, National Cancer Center Hospital; #Division of Translational Genomics, Exploratory Oncology Research & Clinical Trial Center; **National Cancer Center; ‡‡Department of Pathology, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome; ¶Department of Clinical Genomics, National Cancer Center Research Institute, Tokyo, Japan; †Pathology Department, Instituto Valenciano de Oncología, Valencia, Spain; §Unità Operativa Complessa (U.O.C.) Azienda Socio Sanitaria Territoriale Centro Specialistico Ortopedico Traumatologico Gaetano Pini-CTO, Milano, Italy; ‖Department of Anatomical and Cellular Pathology, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong, SAR, China; and §§Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, NY.

Present address: Ken-ichi Yoshida, MD, Department of Pathology, Nihon University School of Medicine, Tokyo, Japan.

Conflicts of Interest and Source of Funding: Supported in part by the JSPS Grant-in-Aid for Young Scientists under award number 18K15108 (A.Y.) and the Cancer Center Support Grant of the National Institutes of Health/National Cancer Institute under award number P30 CA008748 (C.R.A.), P50 CA217694 (C.R.A.), and P50 CA140146-01 (C.R.A.). The remaining authors have disclosed that they have no significant relationships with, or financial interest in, any commercial companies pertaining to this article.

Correspondence: Akihiko Yoshida, MD, PhD, Department of Diagnostic Pathology, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan (e-mail: akyoshid@ncc.go.jp).



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Pseudocarcinomatous Squamous Hyperplasia Involving Bone

Andrew E. Rosenberg, MD*
Smiljana Spasić, MD†
*Department of Pathology and Laboratory Medicine, University of Miami, Miller School of Medicine, Miami, FL
†Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, MA

In Reply:

We appreciate the comments of Ide and colleagues and are thankful that they called attention to this oversight. Our evaluation of the literature did not include experimental animal studies as they are not directly comparable to the findings in the human specimens, and abstracts and reference textbooks were excluded because they are not peer review. However, we failed to identify articles that mentioned pseudoepitheliomatous hyperplasia as a component of medication or radiation induced osteonecrosis of the jaw and, therefore, underestimated the number of cases previously documented.[1–5] We are responsible and apologize for this inadvertent oversight. Of note, none of the published articles that were not included in our evaluation provides the detailed description of pseudocarcinomatous hyperplasia, or the direct comparison with and distinction from squamous cell carcinoma, that our study documents.[6] In contrast, they offer only a brief morphological description and merely state that pseudoepitheliomatous hyperplasia may be present in these conditions, or that squamous epithelium can surround pieces of necrotic bone during the process of transmucosal elimination, or pseudoepitheliomatous hyperplasia can be confused with squamous cell carcinoma. The study by Everett et al[7] provides more histologic information, but the conclusions are limited. We provide evidence based clinicopathological criteria to accurately identify pseudocarcinomatous hyperplasia and importantly, distinguish it from squamous cell carcinoma. This diagnostic problem is a common challenge in this select area of surgical pathology.

Conflicts of Interest and Source of Funding: The authors have disclosed that they have no significant relationships with, or financial interest in, any commercial companies pertaining to this article.

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Confirmation of NKX3-1 Expression in *EWSR1-NFATC2* Sarcoma and Mesenchymal Chondrosarcoma Using Monoclonal Antibody Immunohistochemistry, RT-PCR, and RNA In Situ Hybridization

To the Editor:

In the June issue of *American Journal of Surgical Pathology*, Yoshida et al[1] published immunohistochemical data showing that NKX3-1 (also known as NKX3.1), a widely accepted marker of prostatic adenocarcinoma, was commonly expressed in *EWSR1-NFATC2* sarcoma (9/11 cases) and mesenchymal chondrosarcoma (12/12 cases). Immunostaining was performed using a rabbit polyclonal antibody (Cat No. 0314, dilution 1:500; Athena Enzyme Systems, Baltimore, MD), and the sarcomas exhibited focal or diffuse labeling with variable intensity. They concluded that NKX3-1 could be a useful ancillary tool in sarcoma diagnosis as it was largely unexpressed in histologic mimics of these 2 tumor types.

However, within several months of their publication, 2 groups of researchers reported that these findings were not reproducible. Perret et al[2] showed that all 3 *EWSR1-NFATC2* sarcomas they tested were negative for NKX3-1, using a monoclonal antibody (EP356, prediluted; Bio SB, Santa Barbara, CA). Likewise, Chen

Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website, www.ajsp.com.

HEY1-NCOA2 Fusion Drives MC

GENES, CHROMOSOMES & CANCER 51:127–139 (2012)

Identification of a Novel, Recurrent *HEY1-NCOA2* Fusion in Mesenchymal Chondrosarcoma Based on a Genome-wide Screen of Exon-level Expression Data

Lu Wang,[1] Toru Motoi,[1] Raya Khanin,[2] Adam Olshen,[3] Fredrik Mertens,[4] Julia Bridge,[5] Paola Dal Cin,[6] Cristina R. Antonescu,[1] Samuel Singer,[7] Meera Hameed,[1] Judith V.M.G. Bovee,[8] Pancras C. W. Hogendoorn,[9] Nicholas Socci,[2] and Marc Ladanyi[1]*

[1]Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, NY
[2]Bioinformatics Core, Memorial Sloan-Kettering Cancer Center, New York, NY
[3]Department of Epidemiology and Biostatistics and Helen Diller Family Comprehensive Cancer Center, University of California in San Francisco, San Francisco, CA
[4]Department of Clinical Genetics, Lund University Hospital, Lund, Sweden
[5]Department of Pathology, University of Nebraska Medical Center, Omaha, NE
[6]Department of Pathology, Brigham and Women's Hospital, Boston, MA
[7]Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, NY
[8]Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands
[9]Human Oncology and Pathogenesis Program, Memorial Sloan-Kettering Cancer Center, New York, NY

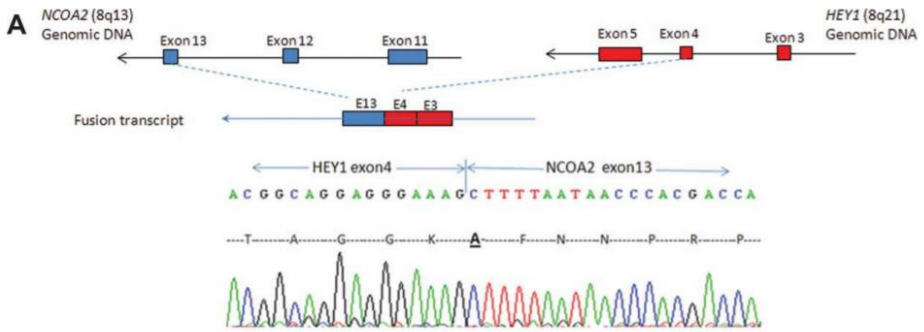
Cancer gene fusions that encode a chimeric protein are often characterized by an intragenic discontinuity in the RNA \expression levels of the exons that are 5′ or 3′ to the fusion point in one or both of the fusion partners due to differences in the levels of activation of their respective promoters. Based on this, we developed an unbiased, genome-wide bioinformatic screen for gene fusions using Affymetrix Exon array expression data. Using a training set of 46 samples with different known gene fusions, we developed a data analysis pipeline, the "Fusion Score (FS) model", to score and rank genes for intragenic changes in expression. In a separate discovery set of 41 tumor samples with possible unknown gene fusions, the FS model generated a list of 552 candidate genes. The transcription factor gene *NCOA2* was one of the candidates identified in a mesenchymal chondrosarcoma. A novel *HEY1-NCOA2* fusion was identified by 5′ RACE, representing an in-frame fusion of *HEY1* exon 4 to *NCOA2* exon 13. RT-PCR or FISH evidence of this *HEY1-NCOA2* fusion was present in all additional mesenchymal chondrosarcomas tested with a definitive histologic diagnosis and adequate material for analysis (n = 9) but was absent in 15 samples of other subtypes of chondrosarcomas. We also identified a *NUP107-LGR5* fusion in a dedifferentiated liposarcoma but analysis of 17 additional samples did not confirm it as a recurrent event in this sarcoma type. The novel *HEY1-NCOA2* fusion appears to be the defining and diagnostic gene fusion in mesenchymal chondrosarcomas. © 2011 Wiley Periodicals, Inc.

INTRODUCTION

Several hundred specific gene fusions have been identified in human cancers, and many of these have emerged as diagnostic or prognostic markers, and some, as therapeutic targets (Mitelman et al., 2007). Sarcomas are a broad and heterogeneous group of cancers, of which at least 50 different histological subtypes have been described. Genetic studies have defined characteristic genetic changes in many sarcoma types, a majority of which were specific gene fusions. Indeed, recurrent, tumor-type specific gene fusions have been identified in about one-third of sarcoma histological subtypes (Mitelman et al., 2007; Mertens et al., 2009). In contrast, in the remaining two-thirds of sarcomas, no characteristic genetic aberrations have been identified.

Historically, the identification of fusion genes has largely been dependent on the detection of structural chromosomal abnormalities by conventional cytogenetic analysis. In recent years, novel gene fusions have been identified based on the detection of cryptic genomic aberrations using new genome-wide screening approaches accompanied by powerful computational data analysis method (Tomlins et al., 2005; Soda et al., 2007; Ozawa et al., 2010).

Additional Supporting Information may be found in the online version of this article.
Supported by: NIH, Grant numbers: P01 CA306450, P01 CA47179
*Correspondence to: Marc Ladanyi, Department of Pathology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065. E-mail: ladanyim@mskcc.org
Received 1 June 2011; Accepted 7 September 2011
DOI 10.1002/gcc.20937
Published online 27 October 2011 in Wiley Online Library (wileyonlinelibrary.com).



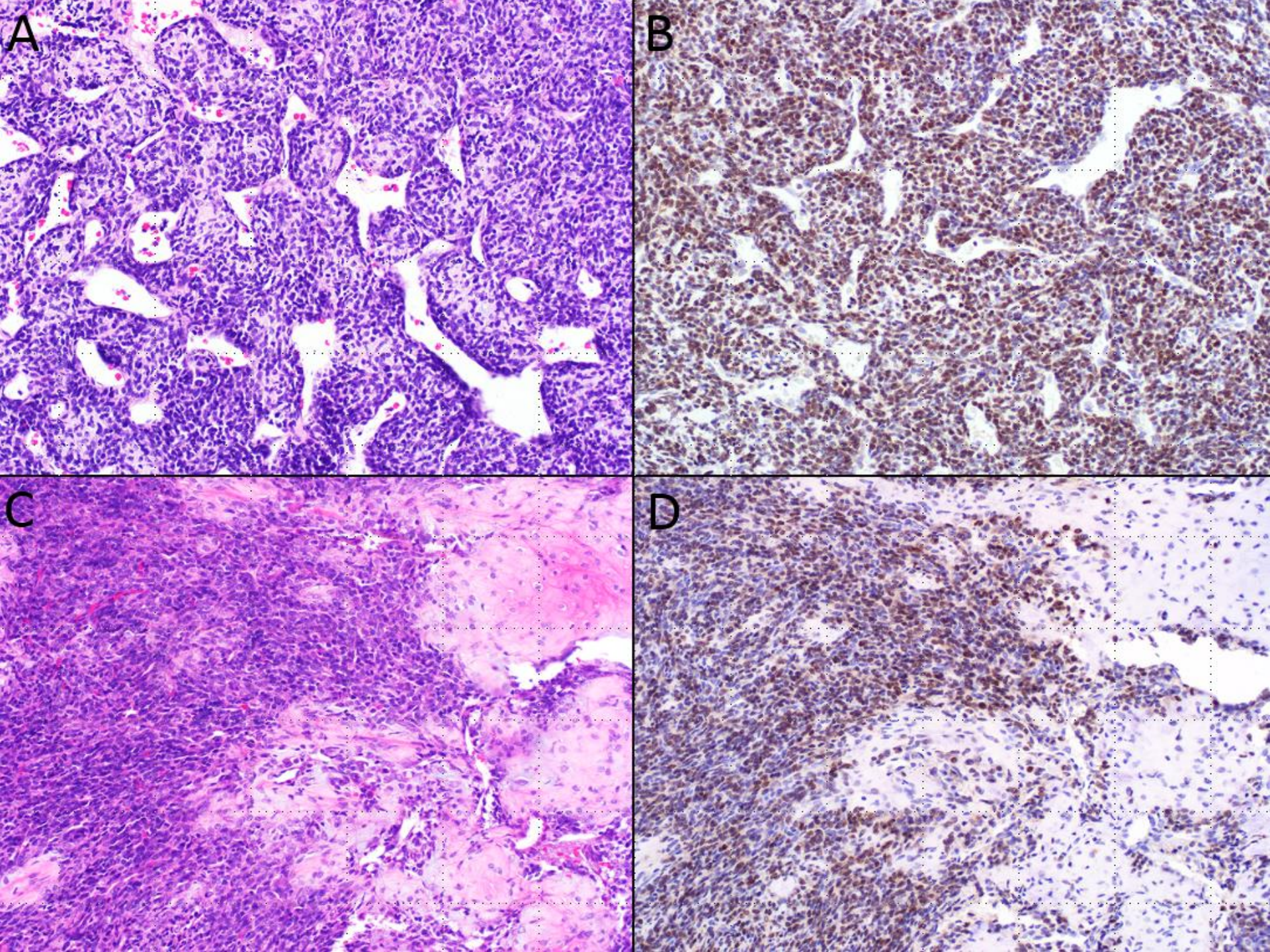
juxtaposes exon 4 of the basic helix-loop-helix (bHLH) transcriptional repressor HEY1 to exon 13 of the steroid receptor coactivator NCOA2

HEY1 ANTIBODY (70R-5242)

Rabbit polyclonal HEY1 antibody raised against the N terminal of HEY1

OVERVIEW

Synonyms	Polyclonal HEY1 antibody, Anti-HEY1 antibody, Hairy/Enhancer-Of-Split Related With Yrpw Motif 1 antibody, CHF2 antibody, HERP2 antibody, HESR1 antibody, HRT-1 antibody, MGC1274 antibody, OAF1 antibody
Specificity	HEY1 antibody was raised against the N terminal of HEY1
Cross Reactivity	Human,Mouse,Rat,Dog
Applications	WB
Immunogen	HEY1 antibody was raised using the N terminal of HEY1 corresponding to a region with amino acids ALGSMSPTTSSQILARKRRRGIIEKRRRDRINNSLSELRRLVPSAFEKQG
Assay Information	HEY1 Blocking Peptide, catalog no. 33R-1335, is also available for use as a blocking control in assays to test for specificity of this HEY1 antibody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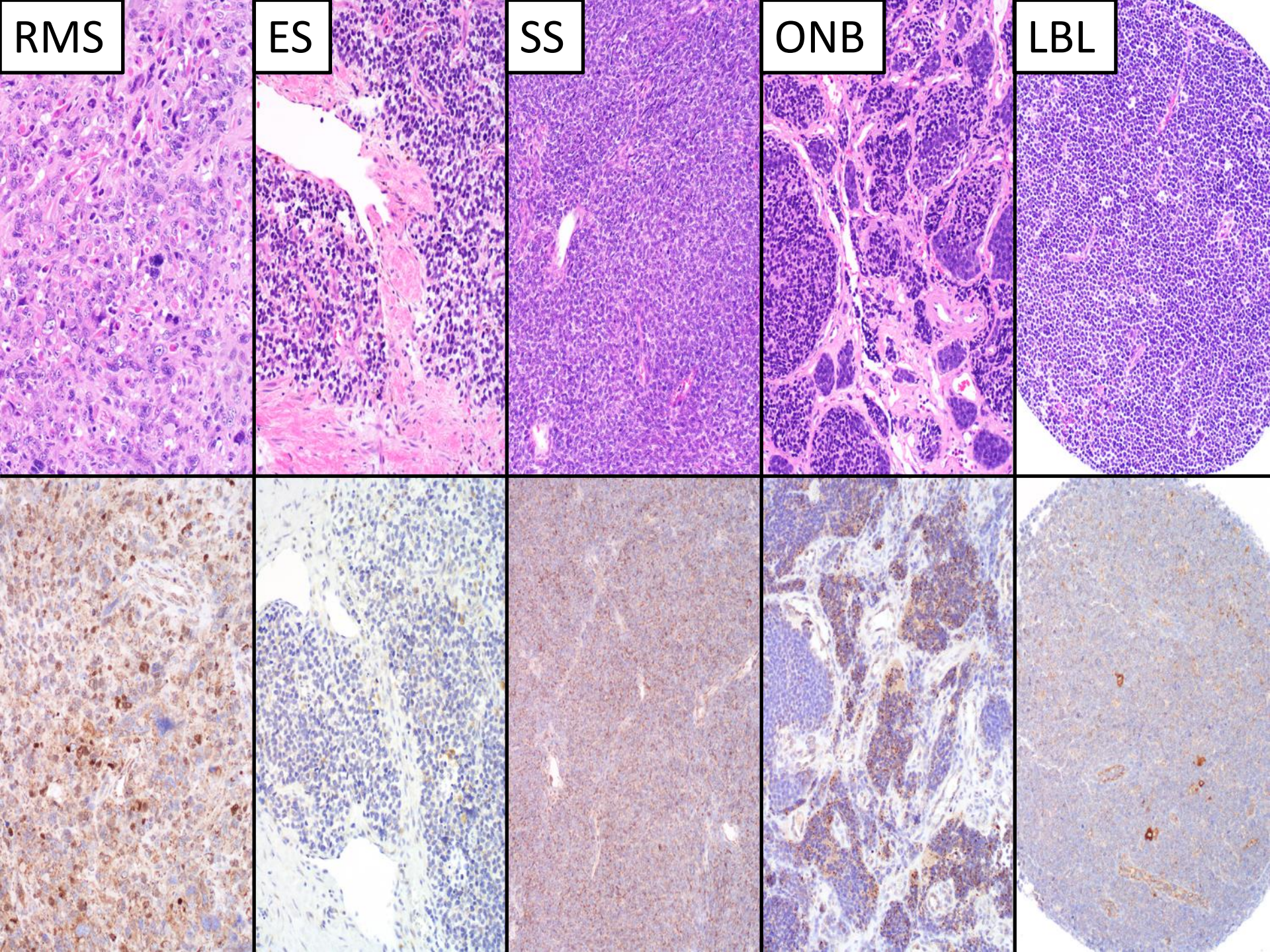
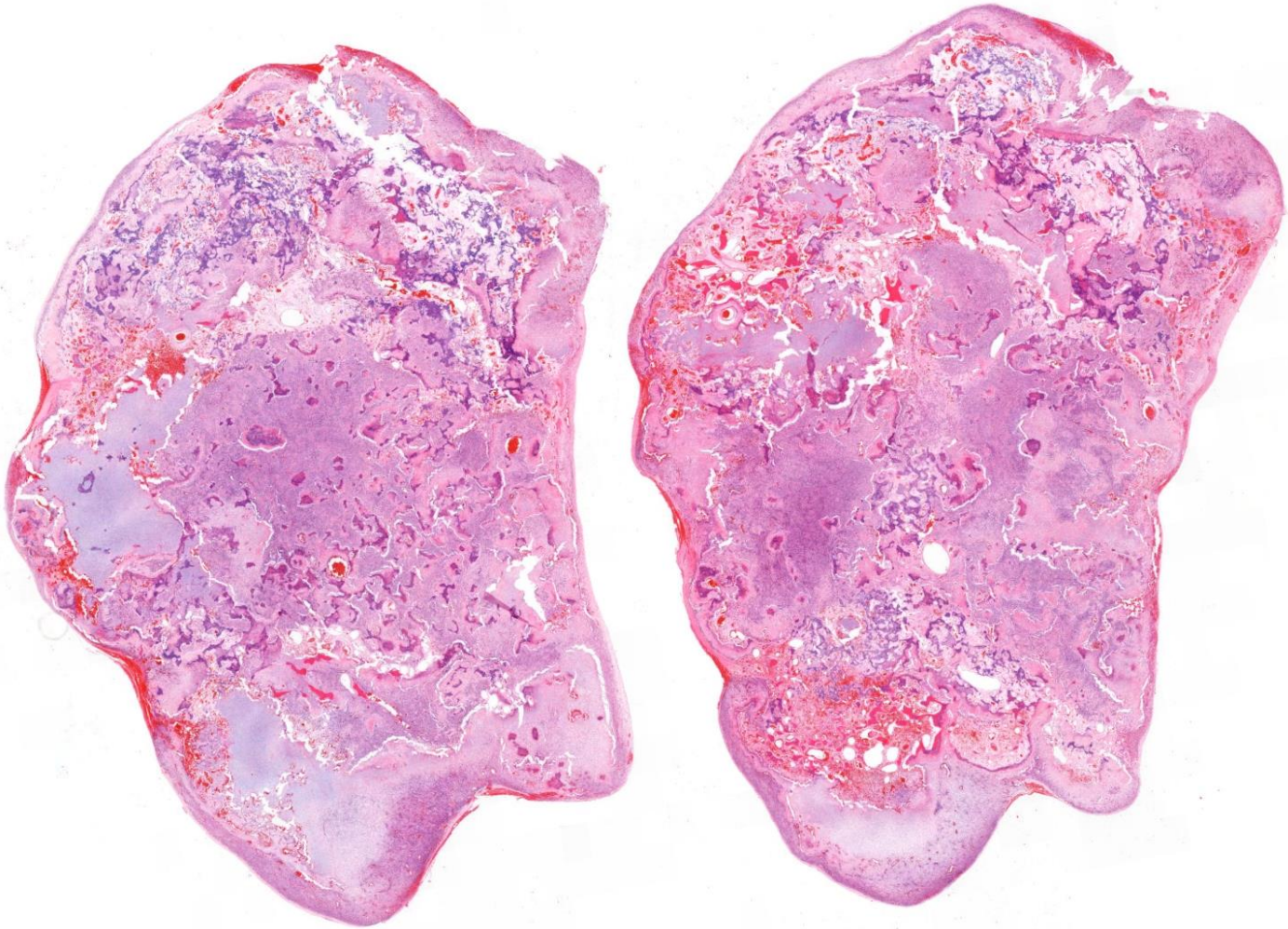

RMS
ES
SS
ONB
LBL

HEY1 and NKX3.1 in SRBCT

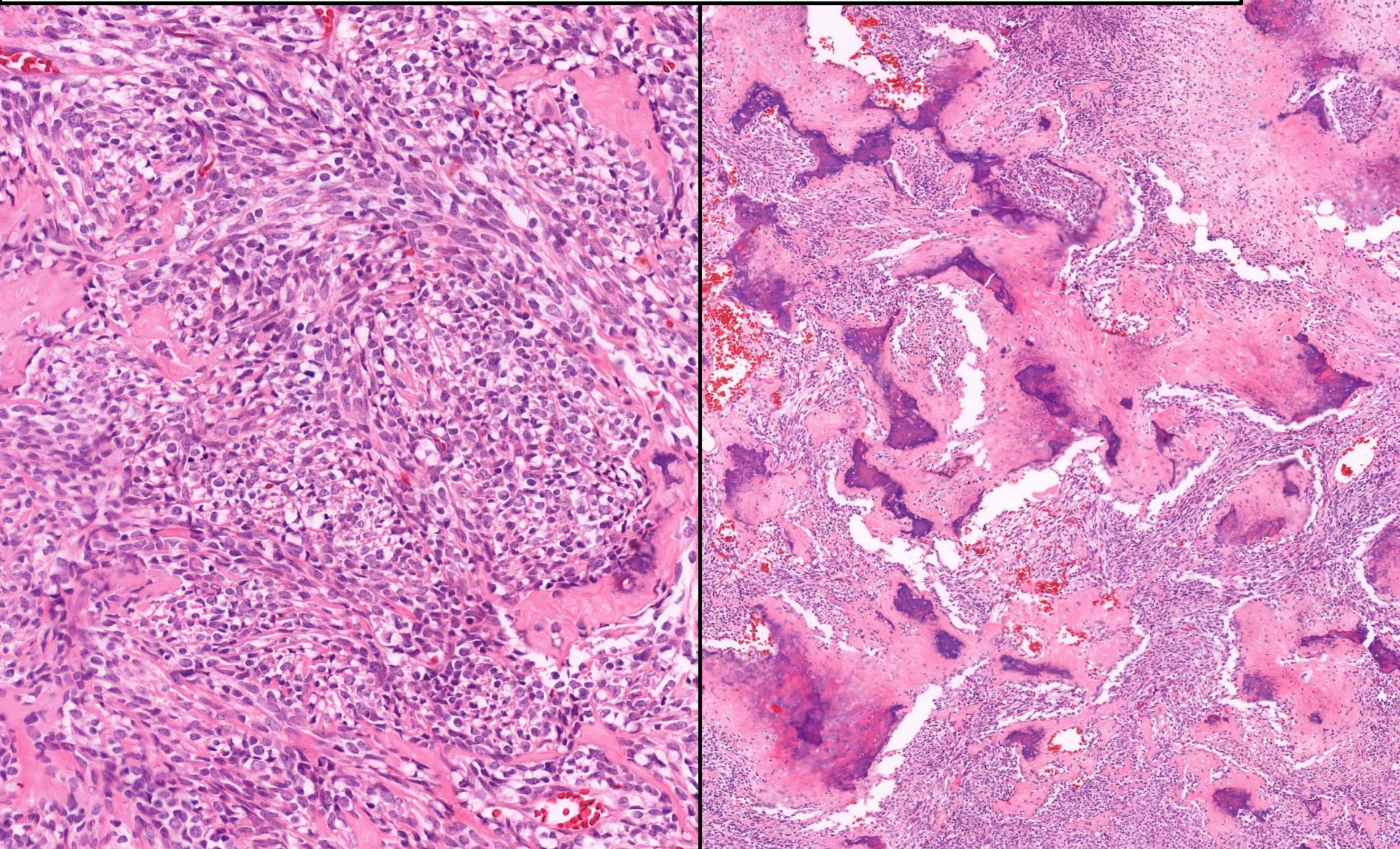
Tumor	# positive/ # total (%)	Mean (Median) H-score (if positive)	H-score range (if positive)
Mesenchymal chondrosarcoma	**29/31 (94%)**	**200 (225)**	**5-300**
Neuroblastoma	0/67 (0)		
Wilms Tumor	0/35 (0)		
Rhabdomyosarcoma	4/23 (17%)	14 (16)	2-22.5
Synovial sarcoma	0/13 (0)		
Ewing sarcoma	0/12 (0)		
BCOR-rearranged sarcoma	0/11 (0)		
Olfactory neuroblastoma	0/10 (0)		
CIC-rearranged sarcoma	0/10 (0)		
Lymphoblastic lymphoma	0/9 (0)		
Desmoplastic small round cell tumor	0/6 (0)		
Cellular congenital mesoblastic nephroma	0/3 (0)		

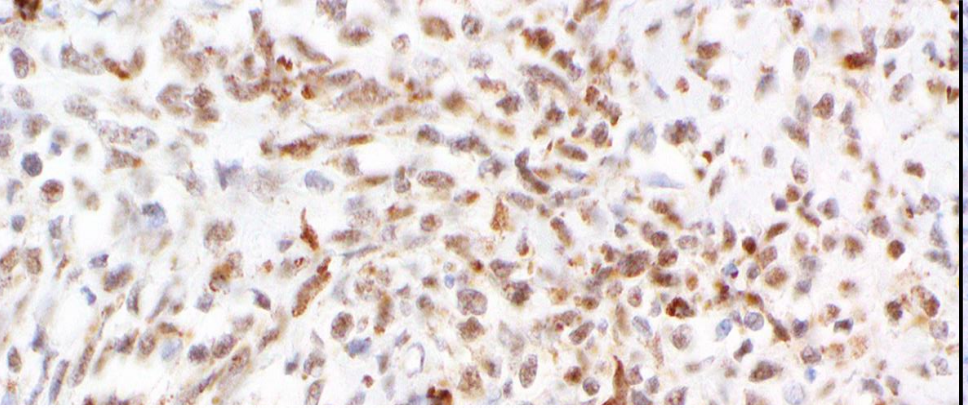
Case Year (Block age in years)	**NKX3.1 H-score**
1981 (39)	1
1982 (38)	0
1993 (27)	1
1995 (25)	1
1995 (25)	10
1996 (24)	15
1996 (24)	70
2000 (20)	30
2016 (4)	120
2018 (2)	80

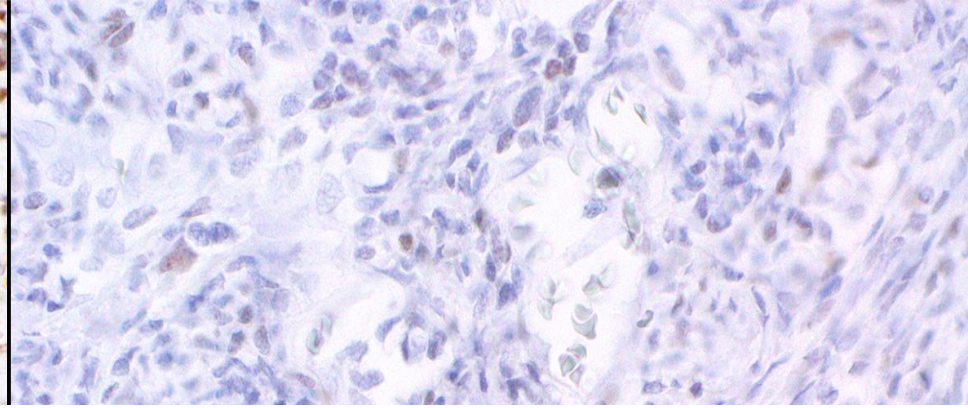


22M with L4-L5 spinal canal tumor; ? association w/ nerve root

MPNST with chondrosarcomatous and osteosarcomatous differentiation





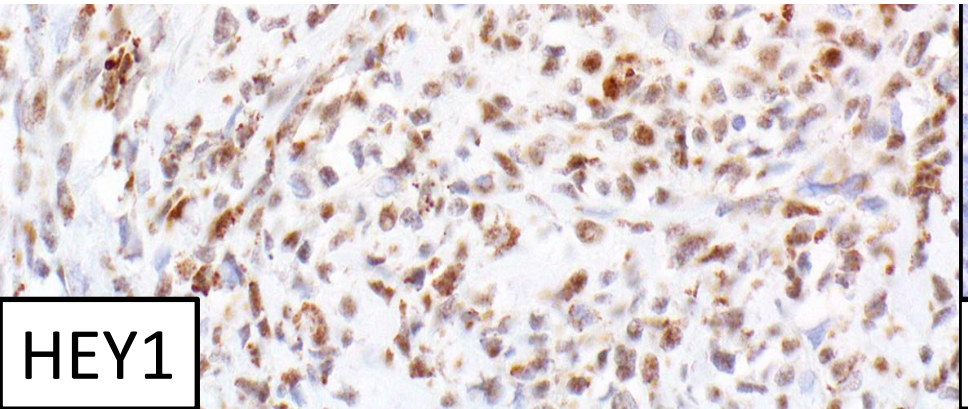


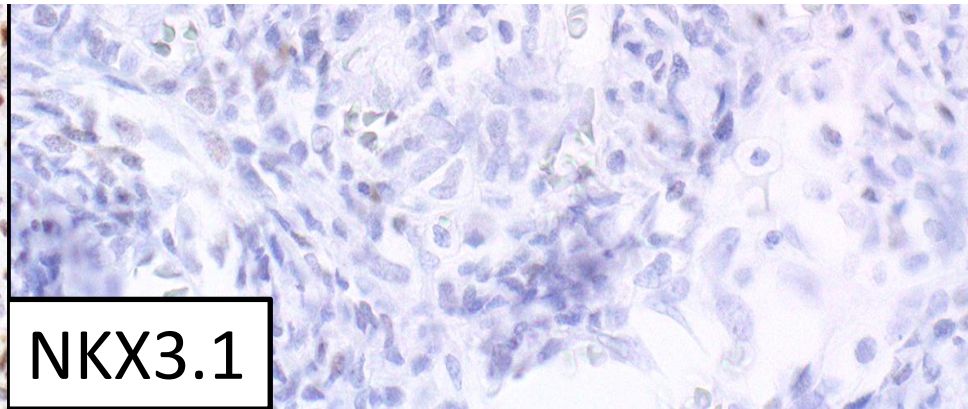
FINAL PATHOLOGIC DIAGNOSIS

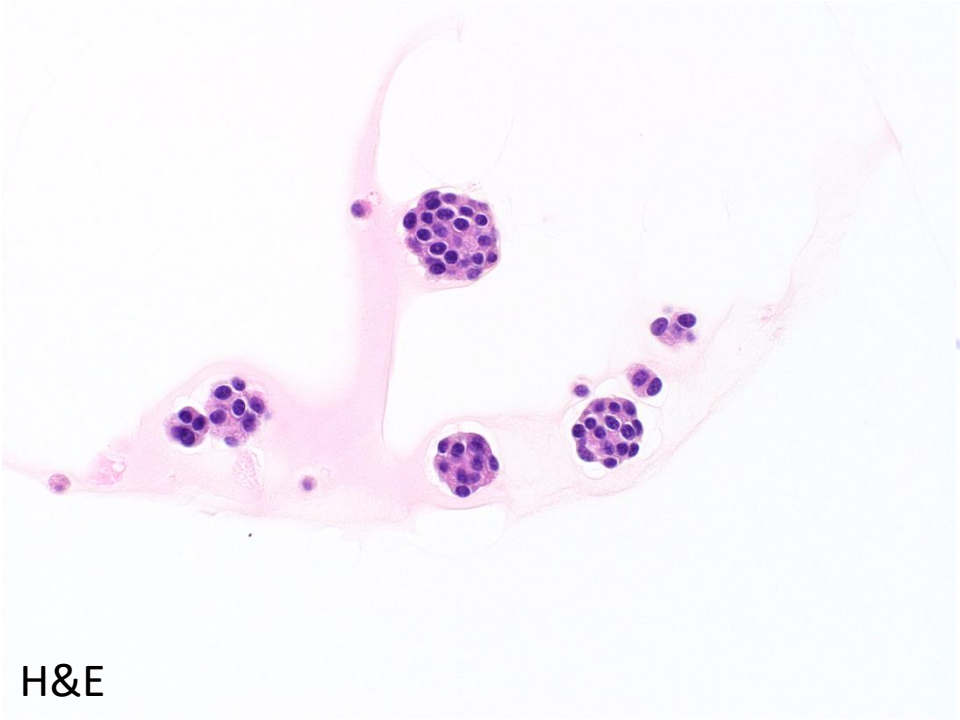
A. SPINAL CORD TUMOR, L4-L5 LEVEL, EXCISION (S21-007125, A1-A5; 02/26/2021; NCS 2779):
Mesenchymal chondrosarcoma, (see note).

Thank you for sharing this interesting case with us. I have studied the glass slides that you submitted and the radiographic images. By imaging, there is a tumor involving the spinal canal that does not show any characteristic features. Histologically, this tumor has different components. It has spindle cell areas, round cell areas, bone, and cartilage. Overall, I felt the histologic features were suspicious for mesenchymal chondrosarcoma, but other tumors were in the differential diagnosis. Immunohistochemical stains done in our laboratory show positive staining for CD99 but no staining for NKX2.2, NKX3.1, Muc4, or Stat6. H3k27me3 is retained (positive nuclear staining). The image you emailed me of HEY1 shows diffuse nuclear staining supporting the diagnosis of mesenchymal chondrosarcoma. I do not have any experience with the HEY1 stain, and it might be worth confirming the diagnosis genetically.

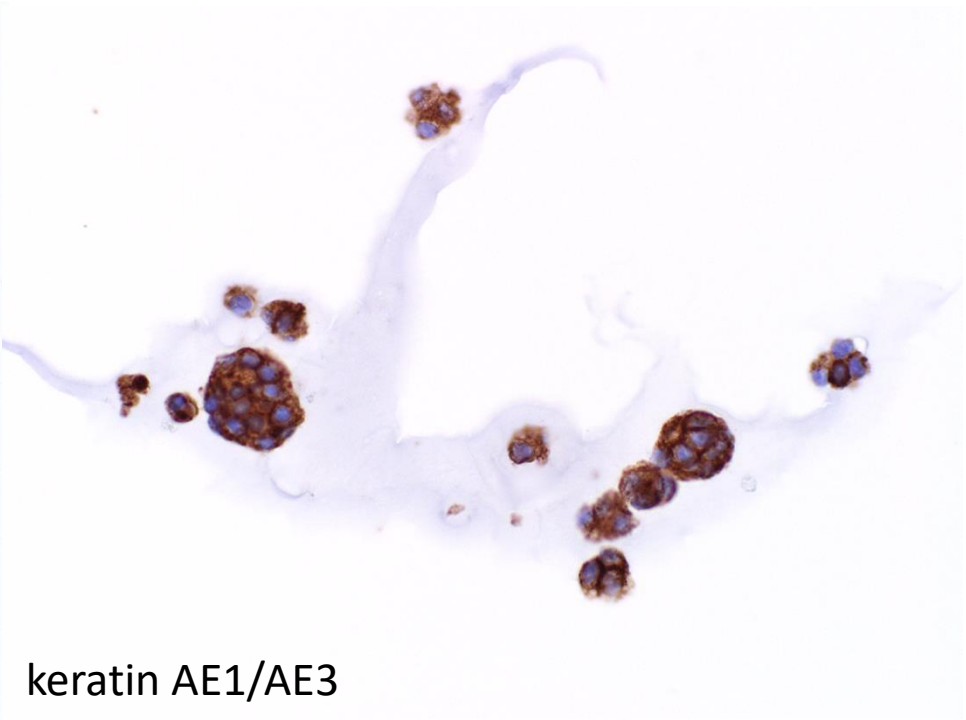
Electronically Signed Out By Gunnlaugur Petur Nielsen MD



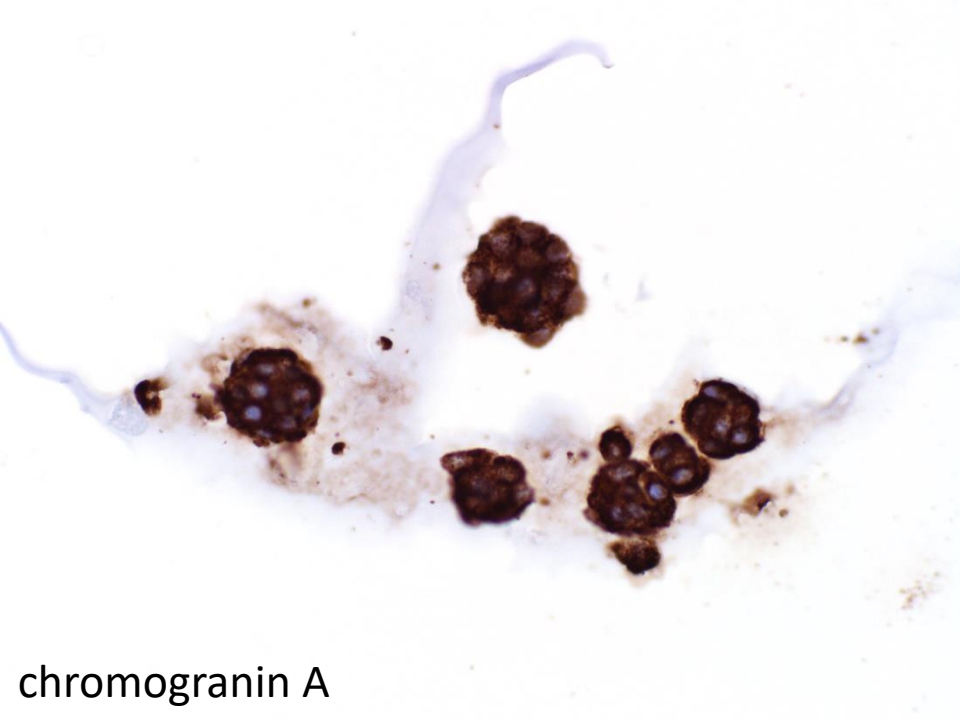




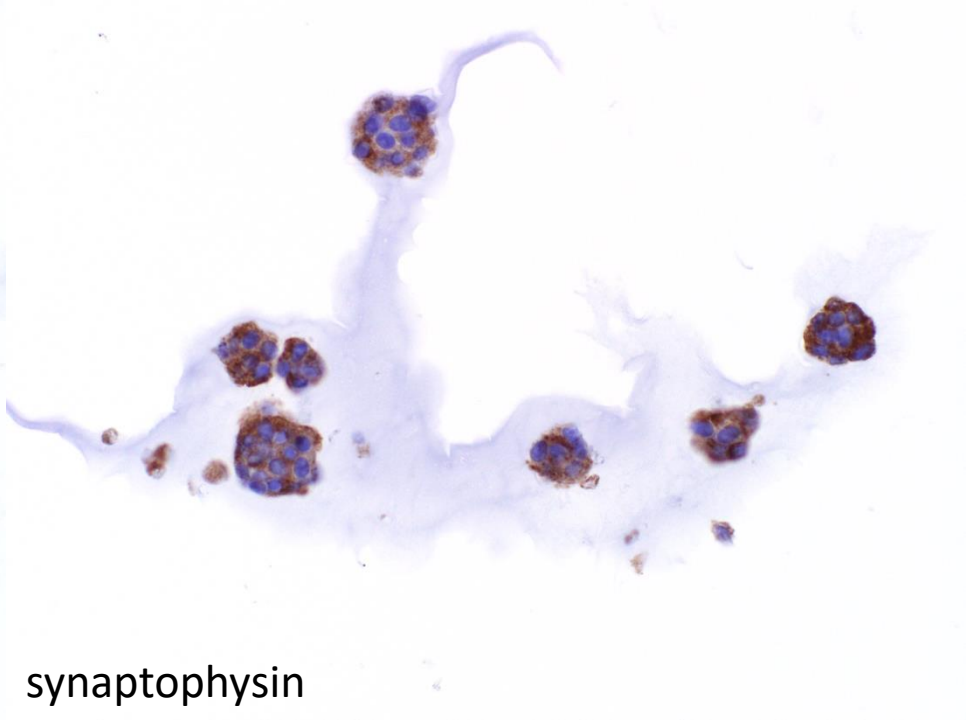
H&E



keratin AE1/AE3



chromogranin A



synaptophysin

BMP Signaling: HIO→HCO

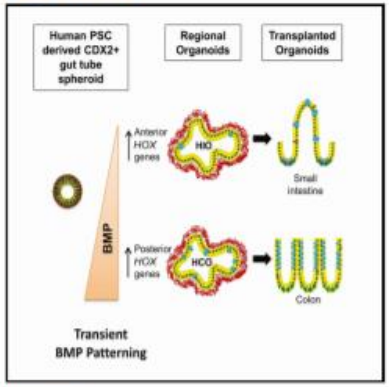
Inducible Intestine-Specific GATA-4 knockout

Article

Cell Stem Cell

Differentiation of Human Pluripotent Stem Cells into Colonic Organoids via Transient Activation of BMP Signaling

Graphical Abstract



Authors

Jorge O. Múnera, Nambirajan Sundaram, Scott A. Rankin, ..., Aaron M. Zorn, Michael A. Helmrath, James M. Wells

Correspondence

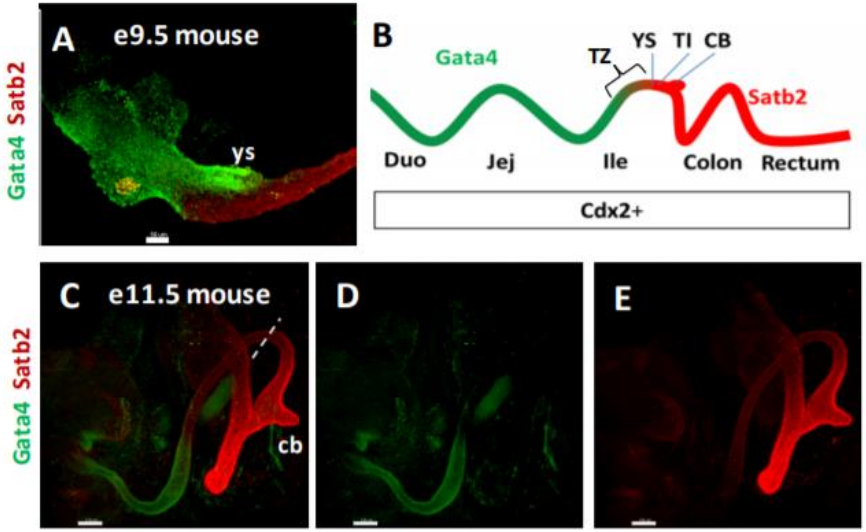
james.wells@cchmc.org

In Brief

Múnera et al. report the generation of pluripotent stem cell (PSC)-derived human colonic organoids (HCOs). They first find that a conserved BMP-*HOX* pathway is required to establish posterior identity and then show that activating BMP signaling in human PSC-derived CDX2+ gut tube spheroids generates HCOs that retain colonic identity after transplantation.

Highlights

- Satb2 expression marks the presumptive large intestine in frog and mouse embryos



Molecular and Cellular Biology, Dec. 2006, p. 9060–9070
0270-7306/06/$08.00+0 doi:10.1128/MCB.00124-06
Vol. 26, No. 23

Gata4 Is Essential for the Maintenance of Jejunal-Ileal Identities in the Adult Mouse Small Intestine

Tjalling Bosse,[1] Christina M. Piaseckyj,[2] Ellen Burghard,[1] John J. Fialkovich,[2] Satish Rajagopal,[3] William T. Pu,[3] and Stephen D. Krasinski[2,4]*

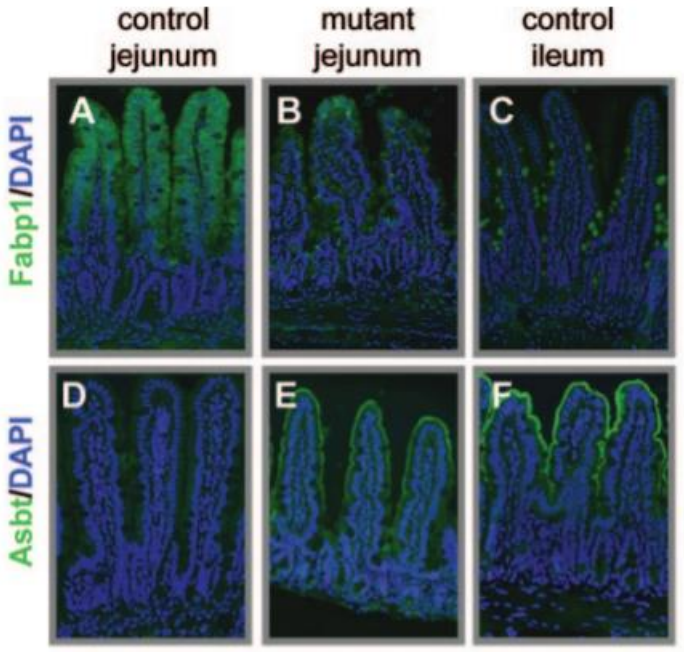
Department of Medicine, University of Amsterdam, Amsterdam 1100DD, The Netherlands[1]; Departments of Medicine[2] and Cardiology,[3] Children's Hospital and Harvard Medical School, Boston, Massachusetts 02115; and Dorothy R. Friedman School of Nutrition Science and Policy, Tufts University, Boston, Massachusetts 02111[4]

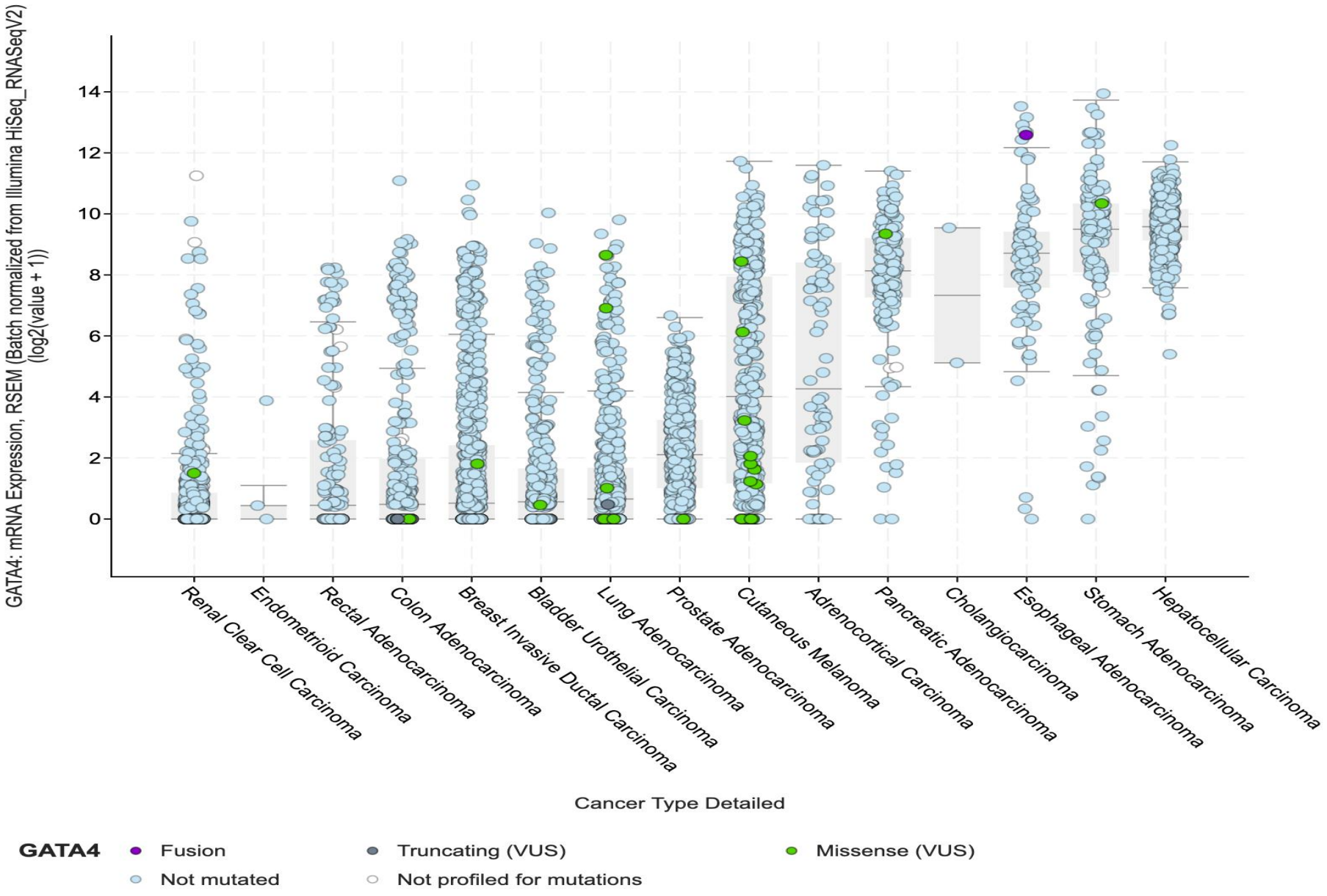
Received 20 January 2006/Returned for modification 17 March 2006/Accepted 22 August 2006

Gata4, a member of the zinc finger family of GATA transcription factors, is highly expressed in duodenum and jejunum but is nearly undetectable in distal ileum of adult mice. We show here that the caudal reduction of Gata4 is conserved in humans. To test the hypothesis that the regional expression of Gata4 is critical for the maintenance of jejunal-ileal homeostasis in the adult small intestine in vivo, we established an inducible, intestine-specific model that results in the synthesis of a transcriptionally inactive *Gata4* mutant. Synthesis of mutant Gata4 in jejuna of 6- to 8-week-old mice resulted in an attenuation of absorptive enterocyte genes normally expressed in jejunum but not in ileum, including those for the anticipated targets liver fatty acid binding protein (*Fabp1*) and lactase-phlorizin hydrolase (*LPH*), and a surprising induction of genes normally silent in jejunum but highly expressed in ileum, specifically those involved in bile acid transport. Inactivation of *Gata4* resulted in an increase in the goblet cell population and a redistribution of the enteroendocrine subpopulations, all toward an ileal phenotype. The gene encoding Math1, a known activator of the secretory cell fate, was induced ~75% ($P < 0.05$). Gata4 is thus an important positional signal required for the maintenance of jejunal-ileal identities in the adult mouse small intestine.

The mammalian small intestine is lined by a highly specialized epithelium that displays a wide-ranging yet tightly regulated functional diversity along its cephalo-caudal axis (13). The functional diversity is linked to a continuous renewal process in which stem cells located near the base of crypts produce transit-amplifying cells that ultimately differentiate into four principal cell types. Absorptive enterocytes, which constitute the majority of intestinal epithelial cells, goblet cells, and en-

Gata4 is a member of an evolutionarily conserved family of zinc finger transcription factors that are necessary for a variety of developmental processes, including embryonic morphogenesis and cellular differentiation (23). Inactivation of GATA homologues in lower animals, such as *elt-2* in *Caenorhabditis elegans* (11), *serpent* in *Drosophila melanogaster* (28), or *gata4* in zebrafish (15), results in defects in heart development and gastrointestinal organogenesis, demonstrating a conservation

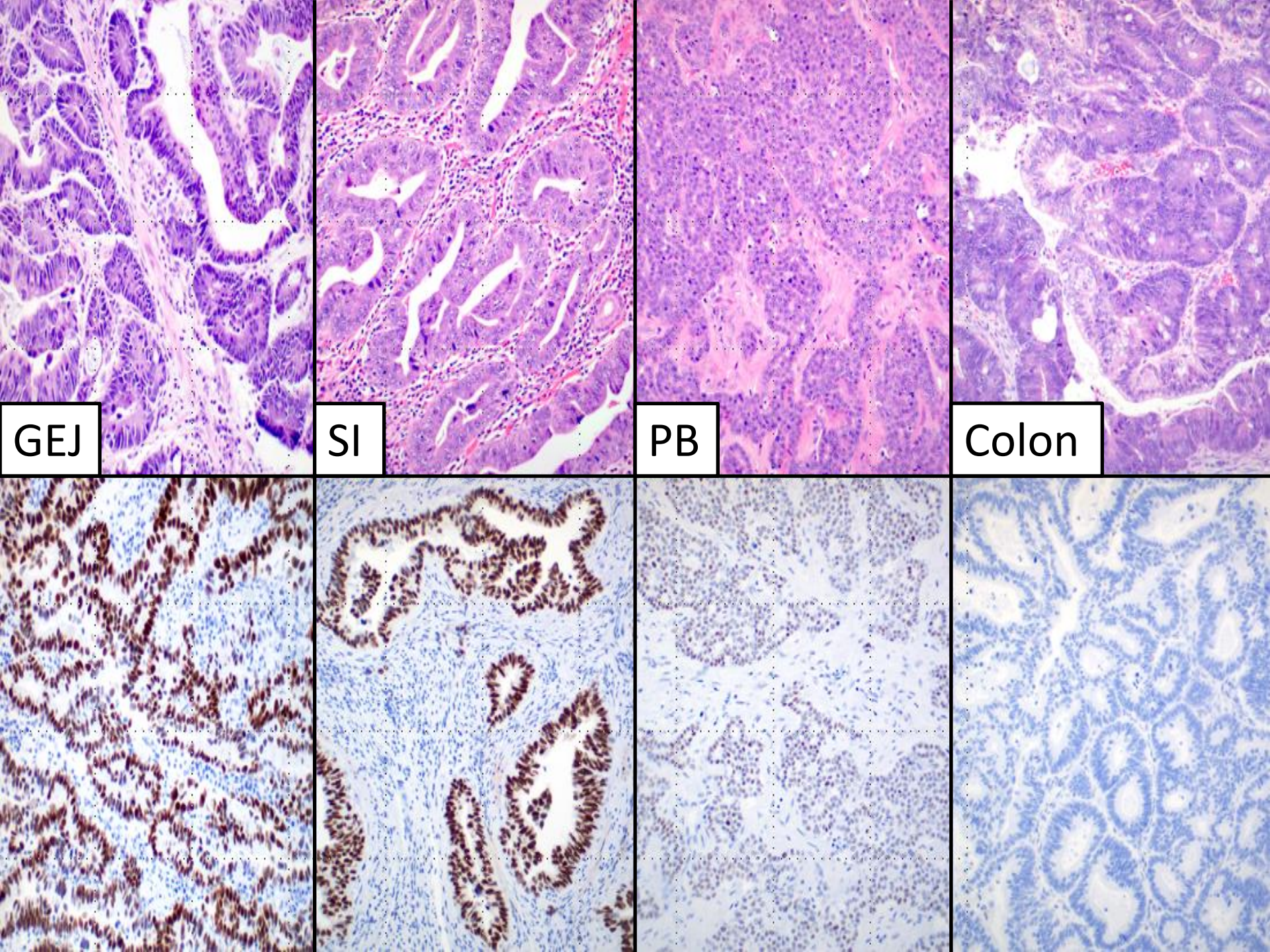


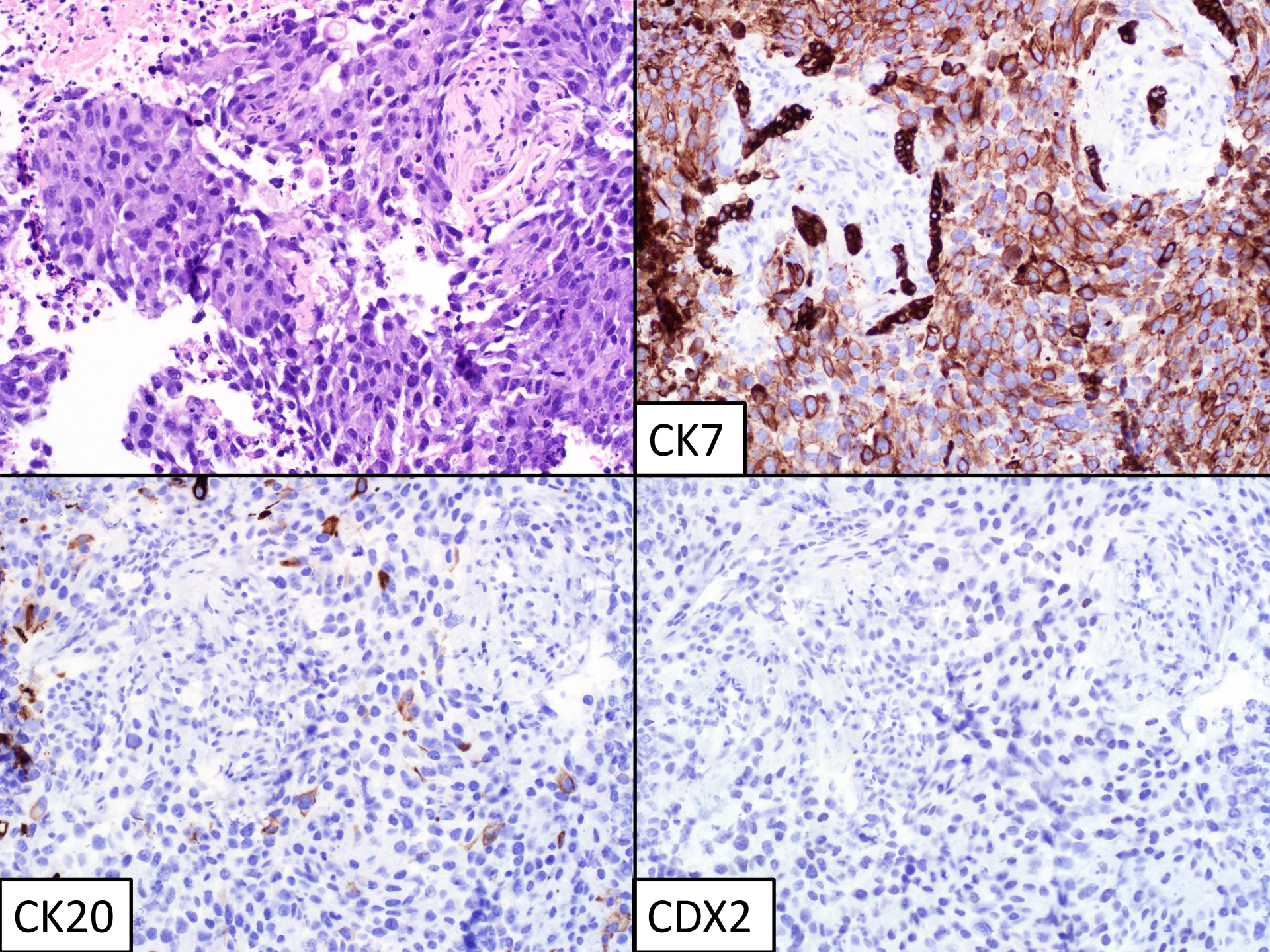

GATA4: mRNA Expression, RSEM (Batch normalized from Illumina HiSeq_RNASeqV2) (log2(value + 1))
14
12
10
8
6
4
2
0
Renal Clear Cell Carcinoma
Endometrioid Carcinoma
Rectal Adenocarcinoma
Colon Adenocarcinoma
Breast Invasive Ductal Carcinoma
Bladder Urothelial Carcinoma
Lung Adenocarcinoma
Prostate Adenocarcinoma
Cutaneous Melanoma
Adrenocortical Carcinoma
Pancreatic Adenocarcinoma
Cholangiocarcinoma
Esophageal Adenocarcinoma
Stomach Adenocarcinoma
Hepatocellular Carcinoma
Cancer Type Detailed
GATA4
Fusion
Truncating (VUS)
Missense (VUS)
Not mutated
Not profiled for mutations

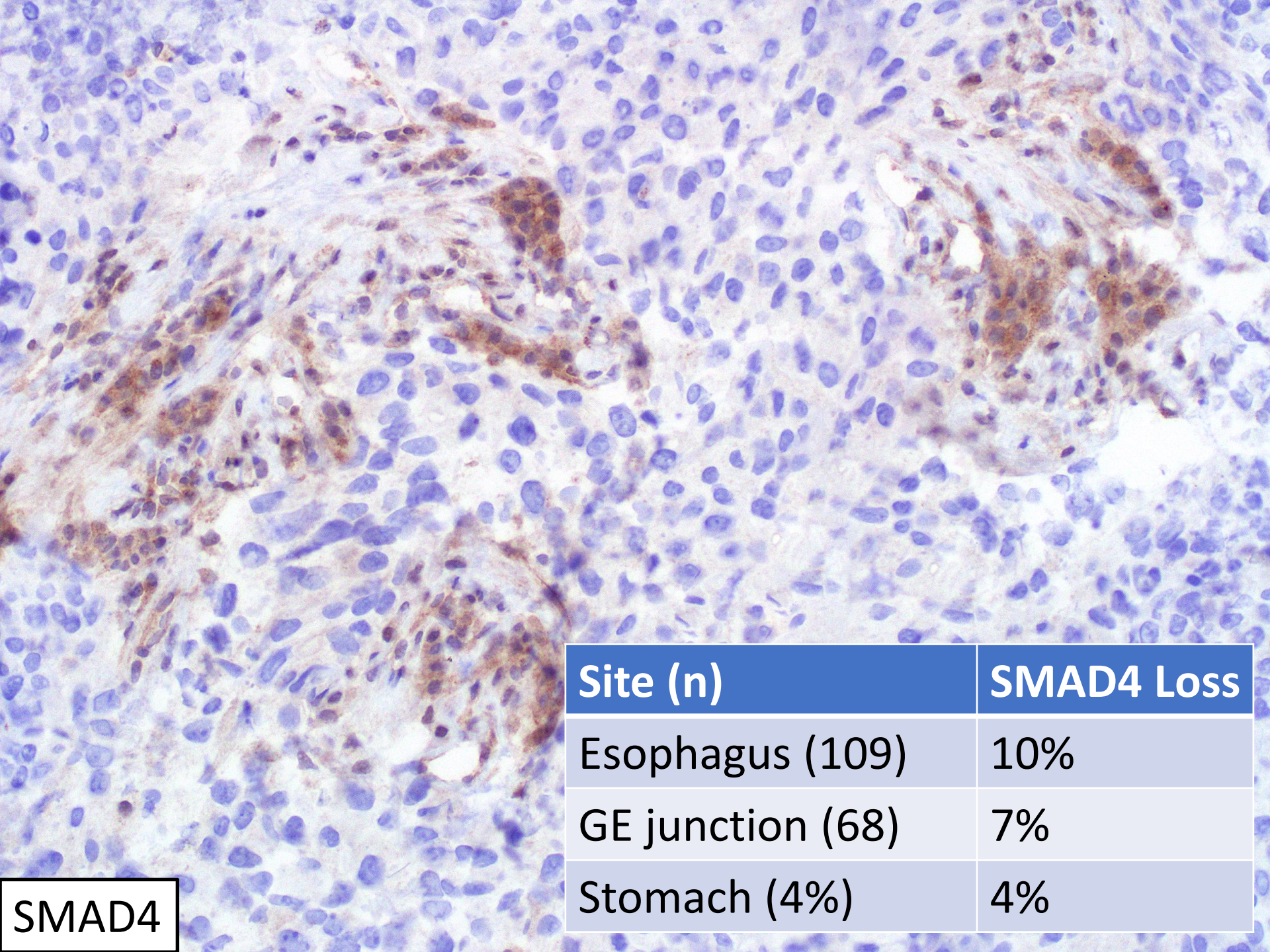
Detailed GATA-4 Results in 1479 Tumors

	Location	Positive (any H score)	Total Number	Median (H if positive)	% H ≥ 50	% H ≥ 100	% H ≥ 200
GI Carcinoma	Stomach	89%	90	235	86%	81%	61%
	Esophagus	77%	129	225	68%	63%	46%
	GEJ	76%	104	195	64%	56%	38%
	Small Bowel Foregut	73%	80	217.5	61%	56%	38%
	Pancreas	69%	273	105	51%	37%	11%
	Hepatocellular	61%	28	165	44%	37%	22%
	Cholangiocarcinoma	56%	134	141.7	40%	32%	19%
	Small Bowel Midgut	53%	15	212.5	33%	33%	33%
	Jejunum	78%	9	215	55%	55%	55%
	Ileum	16%	6	3.3	0%	0%	0%
	Colon	11%	156	70	7%	4%	1%
	Right	19%	68	70	12%	7%	3%
	Left	5%	75	68.3	4%	1%	0%
Other Carcinoma	Mucinous Ovarian	68%	100	260	65%	65%	49%
	Adrenal Cortical	11%	19	129.2	11%	5%	0%
	Non-Mucinous Mullerian	9%	53	32.5	2%	2%	2%
	Neuroendocrine Tumor	8%	48	180	8%	6%	4%
	Renal Cell Carcinoma	6%	33	23.3	0%	0%	0%
	Lung Adenocarcinoma	3%	29	36.7	0%	0%	0%
	Breast	2%	58	22.5	0%	0%	0%
	Thyroid	0%	43	N/A	N/A	N/A	N/A
	Other (Prostate, SCC, Urothelial, NEC)	0%	15	N/A	N/A	N/A	N/A
Epithelioid Nonepithelial Neoplasm	Mesothelioma	38%	13	146.5	23%	23%	15%
	Melanoma	29%	14	95.8	21%	14%	7%
	Pheochromocytoma	5%	22	87.5	5%	0%	0%
	Paraganglioma	0%	23	0	0%	0%	0%

GATA-4 mouse monoclonal ab (clone 532020, R&D Systems)

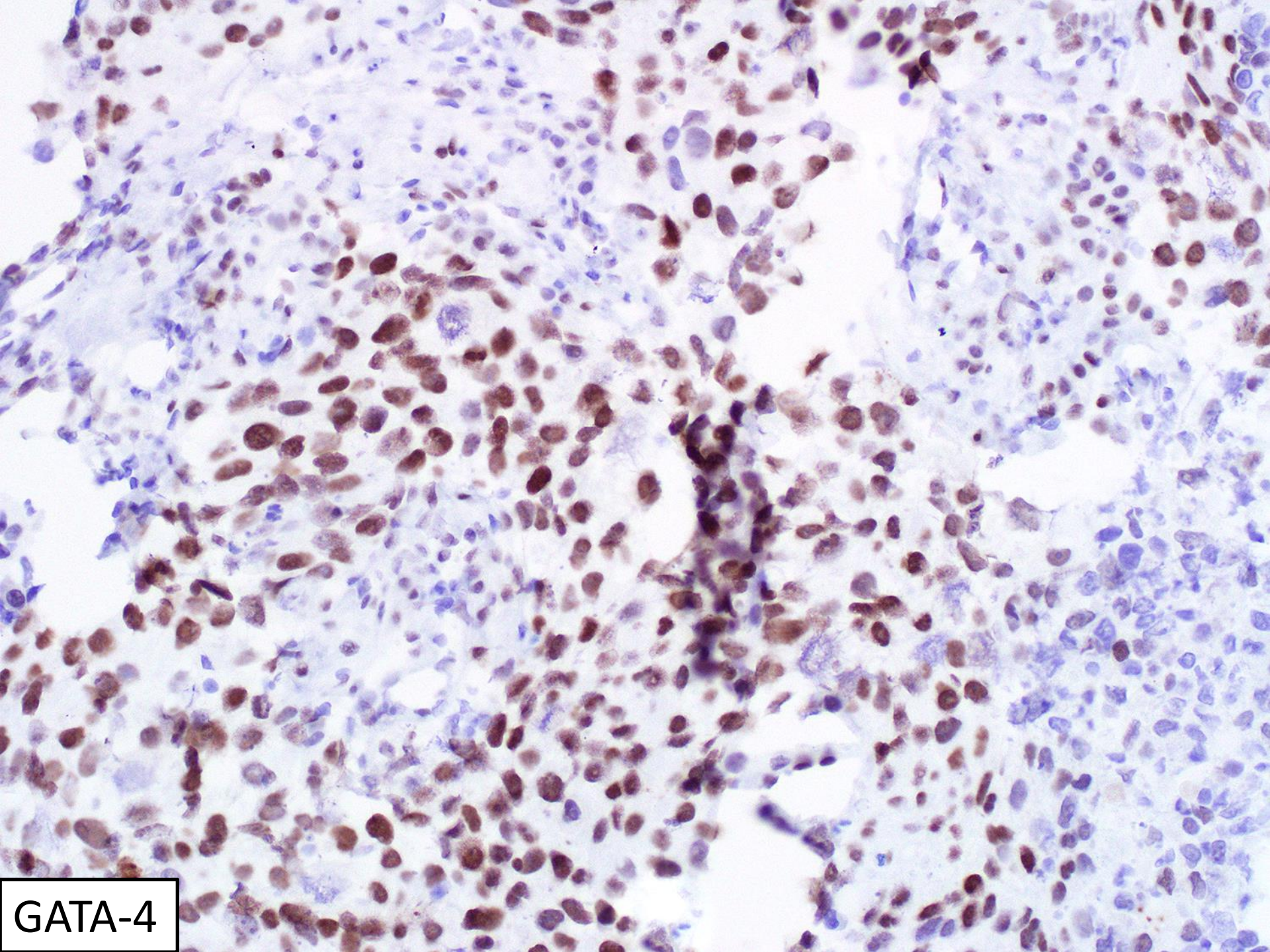

GEJ
SI
PB
Colon


CK7
CK20
CDX2



Site (n)	SMAD4 Loss
Esophagus (109)	10%
GE junction (68)	7%
Stomach (4%)	4%

SMAD4


GATA-4

Diagnostic Utility of GATA-4

1. Distinction of small intestinal from CRC
2. Distinction of upper GI from PR carcinomas
   - Strong GATA-4-positivity favors upper GI origin
3. Non-colonic pan-GI marker, especially useful in PB carcinomas
   - GATA-4-positivity (65%) outpaces CDX2 and CDH17 (~30%)

“A pathologist looking down the barrel of a light microscope at an H&E-stained slide is doing ‘subcortical integrative genomics.’”

Steven Mentzer, MD
Division of Thoracic Surgery
Brigham and Women’s Hospital

Messages Jason Details

Wed, Feb 24, 4:56 PM

Nice! Aidan can lighten your service load...

Give him the polyps to start.