Medizinische Universität Graz

Serrated Polyps

XXVI Meeting of the Adriatic Society of Pathology Split,

30th June - 1st July 2012

ENGIP

Cord Langner MD
Institute of Pathology
Medical University of Graz / Austria

Outline

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- **Pathogenesis of colorectal cancer: adenoma-carcinoma-sequence**
- **Colorectal polyps**
  - Hyperplastic polyp (HP)
  - Traditional serrated adenoma (TSA)
  - Sessile serrated adenoma (SSA)
  - Classical adenoma (TA, TVA, VA)
- **The serrated route: implications for the classification of colorectal cancer**
- **Can immunohistochemistry help to classify serrated lesions?**

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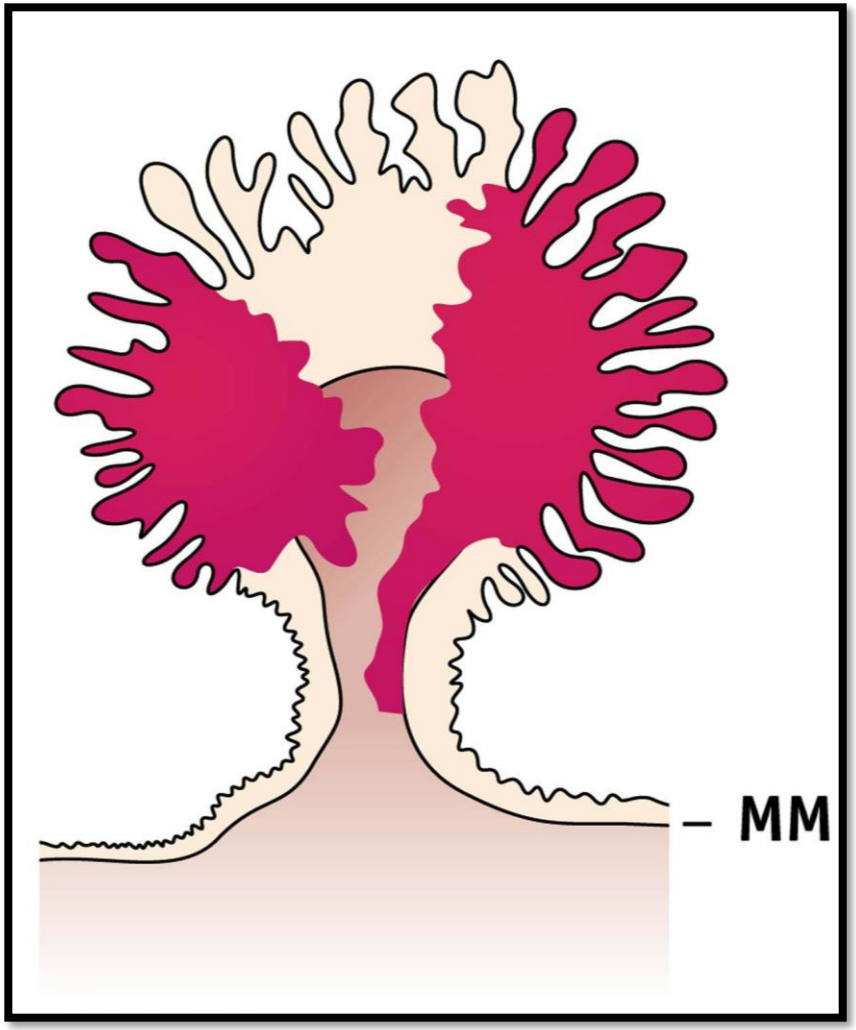
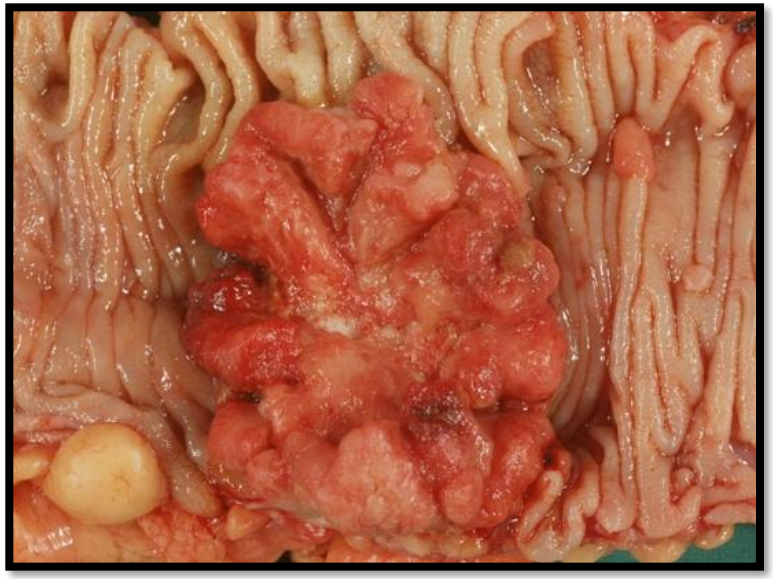
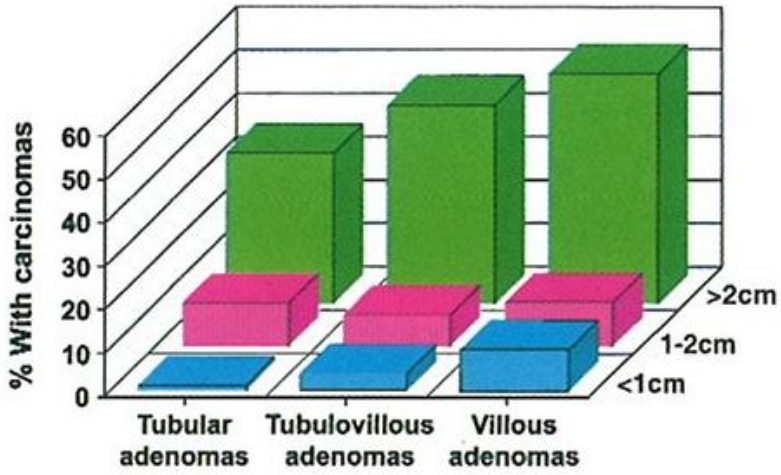
Adenoma-Carcinoma-Sequence



from Böcker, Denk, Heitz – Pathologie, 2004





„Suppressor Phenotype“ of Colorectal Cancer

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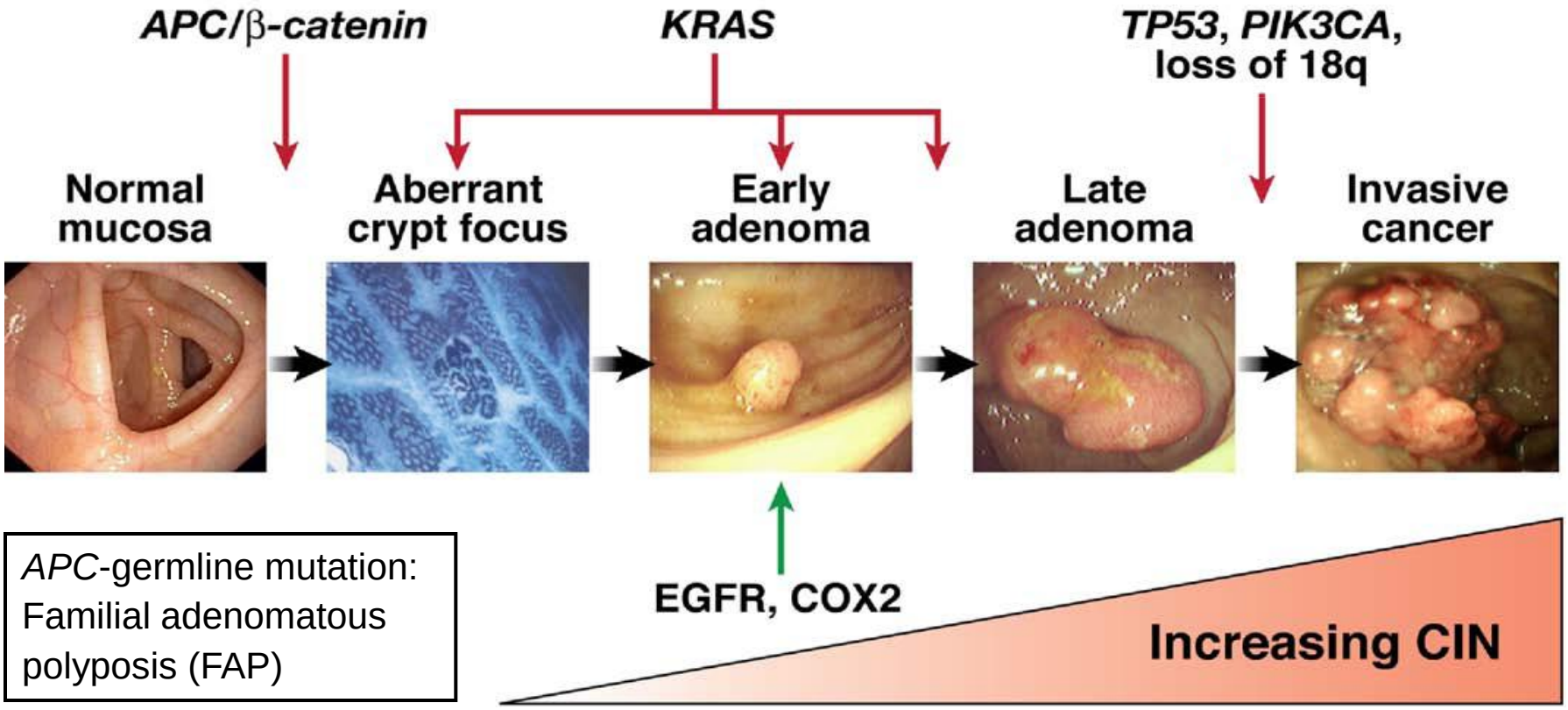
ACF	5%	*APC*-Mutation
Adenoma	30-70%	*APC*-Mutation
Adenocarcinoma	70-80%	*APC*-Mutation

Adenoma	4-26%	*p53*-Mutation
„Malignant Polyp“	50%	*p53*-Mutation
Adenocarcinoma	70-80%	*p53*-Mutation



APC-germline mutation: Familial adenomatous polyposis (FAP)

Fearon und Vogelstein, Cell, 1990
Pino und Chung, Gastroenterology 2010

Lynch-Syndrome (HNPCC-Syndrome)

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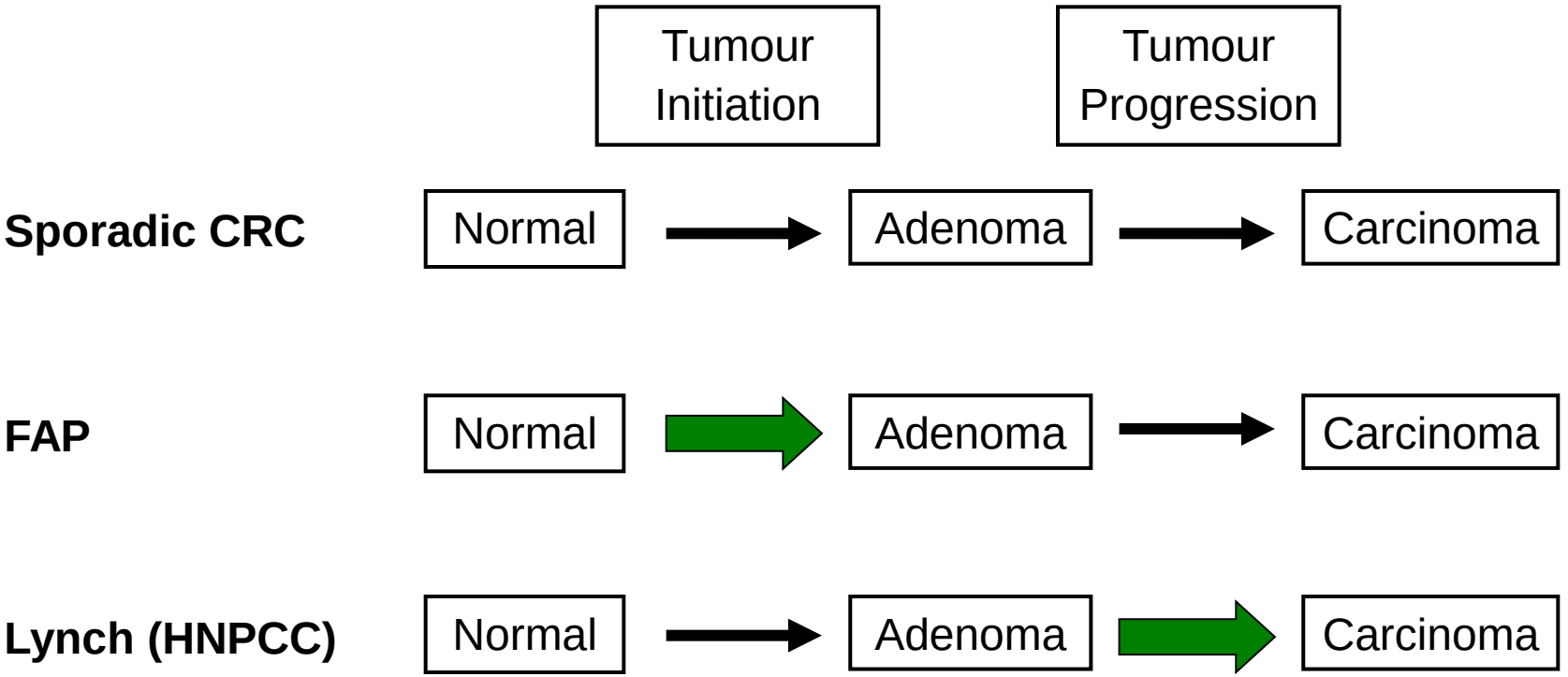
- **Definition**
  - Autosomal dominant disorder caused by a defect in a DNA mismatch repair (MMR) gene (MLH1, MSH2, MSH6, PMS2): microsatellite instability (MSI) and non-corrected mutations in target genes („mutator phenotype“)
- **Clinicopathological Features**
  - Mainly young patients (<50 years)
  - Predominantly right sided tumours often with „non-conventional“ morphology (i.e. mucinous, medullary, signet-ring cell)
  - Associated cancers (endometrium, urothelium, stromach etc.)
- **Diagnosis**
  - **First step:** immunohistochemistry (MMR proteins)
  - **Second step:** molecular analysis (MSI, *BRAF*)

„Suppressor Phenotype“ and „Mutator Phenotype“: Time-Related Differences in Cancer Development



Fearon, Annu Rev Pathol 2011
Langner Pathologe 2011

CpG Island Methylator Phenotype (CIMP)

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- There are CRC that lack both CIN and MSI
- MSI occurs more often in the sporadic setting than in Lynch-Syndrome
- Some CRC are characterized by frequent epigenetic alterations, including aberrant methylation of 5' promoter regions of genes with CpG islands (Cytosin → Methylcytosin)
- Methylation causes transcriptional silencing and can inactivate tumour suppressor genes (e.g. p16, MGMT, **hMLH1 → MSI**)

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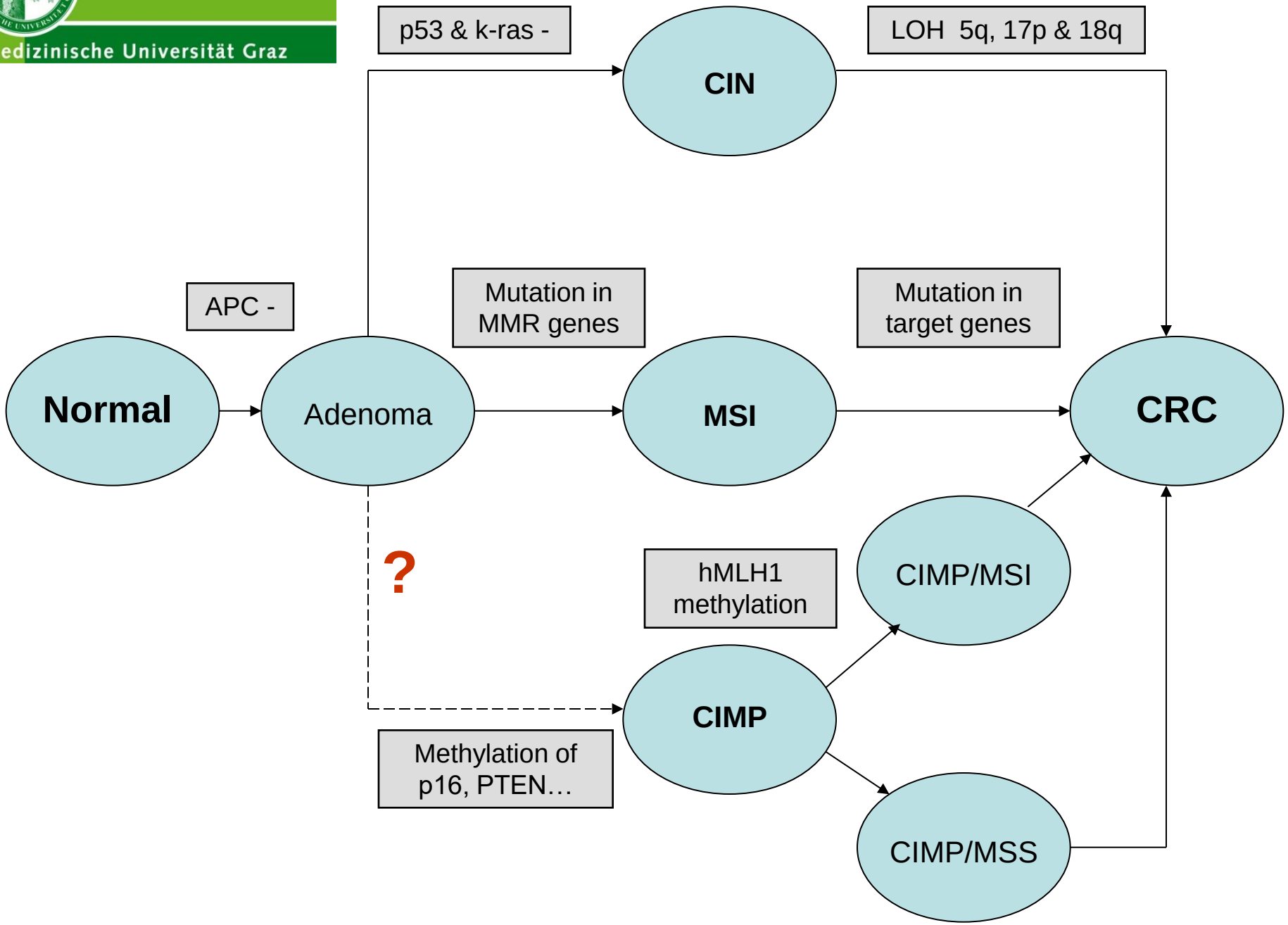

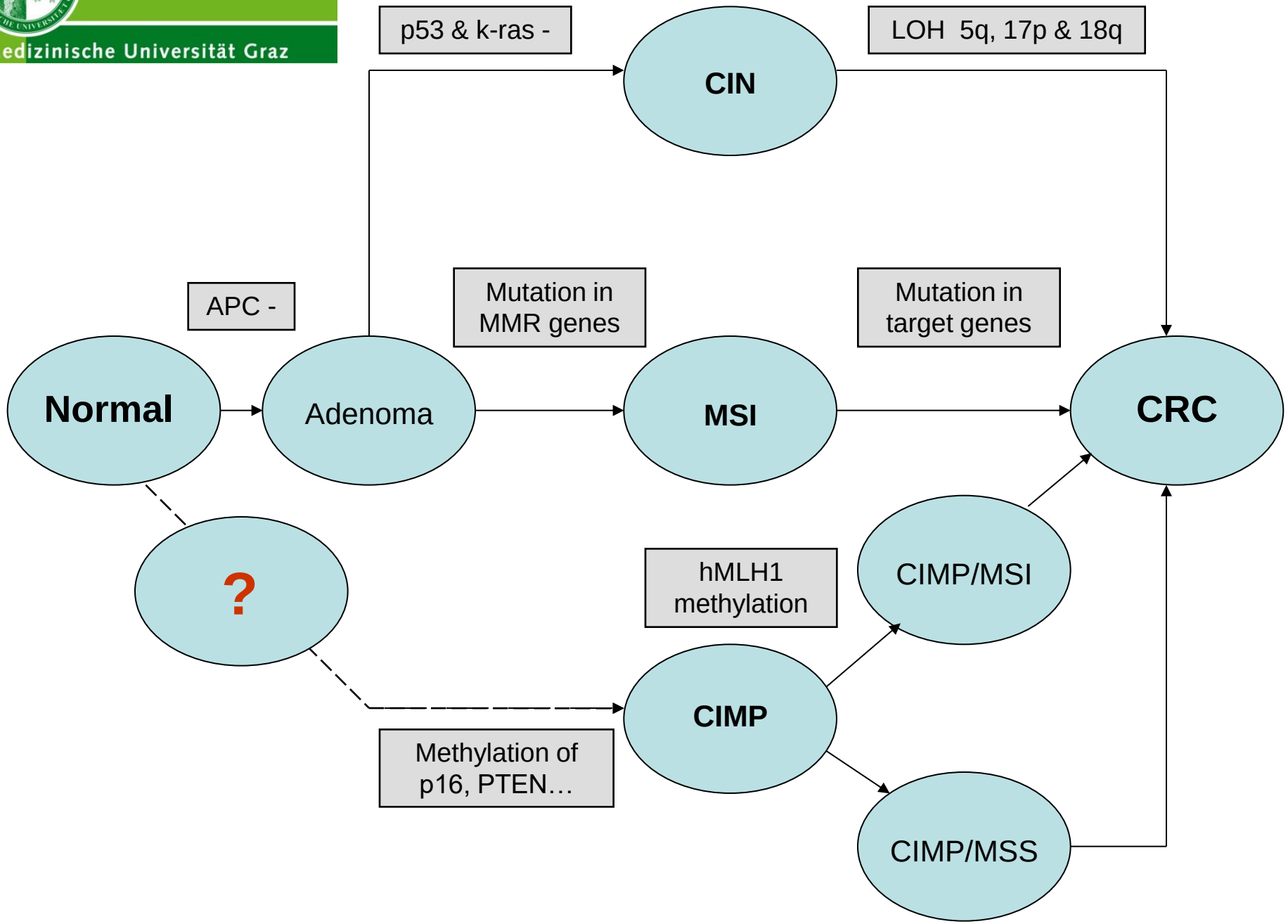
p53 & k-ras -
CIN
LOH 5q, 17p & 18q
APC -
Mutation in MMR genes
Mutation in target genes
Normal
Adenoma
MSI
CRC
?
hMLH1 methylation
CIMP/MSI
CIMP
Methylation of p16, PTEN…
CIMP/MSS

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Serrated Lesions

- The serrated route: implications for the classification of colorectal cancer
- Can immunohistochemistry help to classify serrated lesions?

Hyperplastic Polyp (HP)

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- **Incidence**
  - >75% of serrated polyps
- **Morphology**
  - Location: mainly distal colon
  - Microvesicular subtype (MHVP)
  - Goblet-cell rich subtype (GCHP)
  - Mucin-poor subtype (MPHP)
- **Molecular Pathology**
  - *BRAF* mutation in up to 40% (MVHP)
  - *KRAS* mutation in up to 40% (GCHP)
  - CIMP-phenotype (with methylation of MLH1 und MGMT) may be observed

Hyperplastic Polyp (HP)

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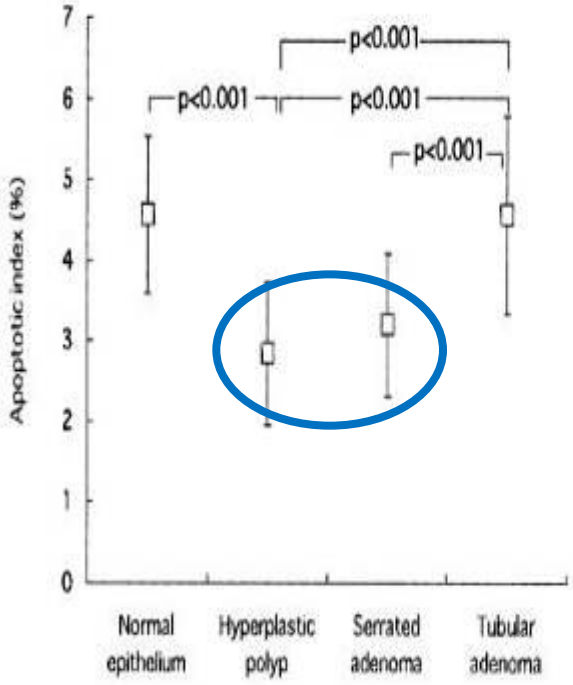
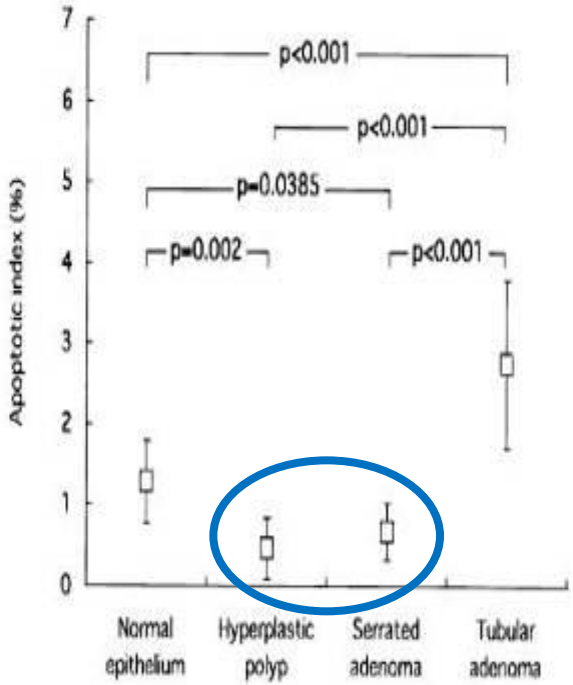
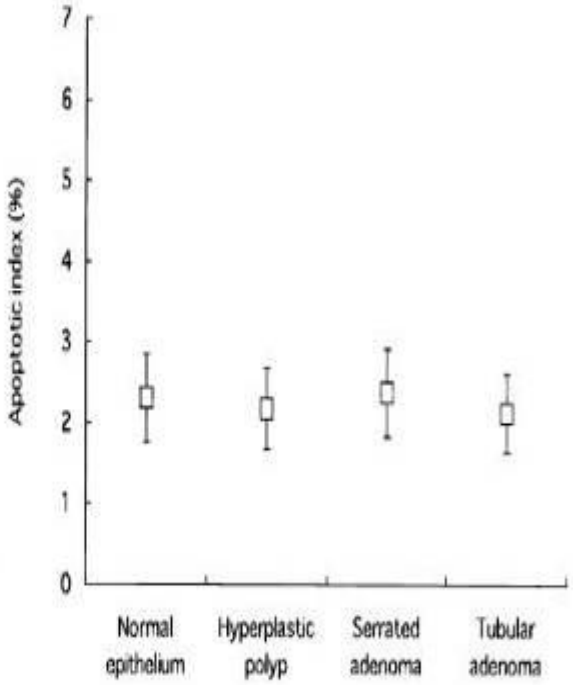
Morphogenesis of Hyperplastic Polyp (HP)



Upper third of the crypt



Central portion of the crypt



Lower third of the crypt

Tateyama H et al. Am J Surg Pathol 2002

Incidence of Hyperplastic Polyp (HP)

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3060 HP in 226/263 (86%) Patients

HP	N	Right Colon
< 6 mm	3020	5%
6-10 mm	38 (8,7%)	42%
>10 mm	2 (0,8%)	100%
Total	3060	

Yano T et al. J Gastroenterol Hepatol 2005

Serrated Polyps with „Abnormal Proliferation“ Part I

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- **Mixed hyperplastic adenomatous polyp - an underdiagnosed entity**

  Report of a case of adenocarcinoma arising within a mixed hyperplastic adenomatous polyp

  - We report a case of colonic adenocarcinoma arising within a polyp with mixed morphology of a hyperplastic polyp and tubular adenoma. Despite the relatively small size of the polyp, two isolated foci of adenocarcinoma in situ were present and tumor islands invaded the submucosa. Isolated areas, morphologically resembling hyperplastic glands, and varying degrees of atypia. **Though rare, some hyperplastic polyps may be precursors of adenomas.**

Urbanski et al. Am J Surg Pathol 1984

Serrated Polyps with „Abnormal Proliferation“ Part II

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- **Mixed hyperplastic adenomatous polyps/serrated adenomas. A distinct form of colorectal neoplasia.**
  - 110 colorectal mixed hyperplastic adenomatous polyps (MHAP) that exhibited the architectural but not the cytologic features of a hyperplastic polyp.
  - MHAP measured 0.2-7.5 cm in diameter. They were distributed throughout the colorectum, but a slight preponderance of large lesions (more than 1.0 cm) occurred in the coecum and appendix.
  - All MHAP were characterized by a serrated glandular pattern simulating that seen in hyperplasia (27% of MHAP were initially diagnosed as hyperplastic polyps).
  - 37% of MHAP contained foci of significant dysplasia, and 11% contained areas of intramucosal carcinoma.

Longacre und Fenoglio-Preiser. Am J Surg Pathol 1990

Traditional Serrated Adenoma (TSA)

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- **Incidence**
  - Rare: <1% of all colorectal polyps
- **Morphology**
  - Location: left > right (distal colon and rectum)
  - Macroscopy / Endoscopy: polypoid lesion
  - **Histology:**
    - Complex villiform growth pattern with prominent serration
    - Ectopic crypt foci (ECF)
    - Cytological dysplasia (intraepthelial neoplasia)
    - Diffuse eosinophilic cytoplasm
- **Molecular Pathology**
  - Poorly defined (due to divergent inclusion criteria): *KRAS* mutation > *BRAF* mutation, CIMP?

Traditional Serrated Adenoma (TSA)

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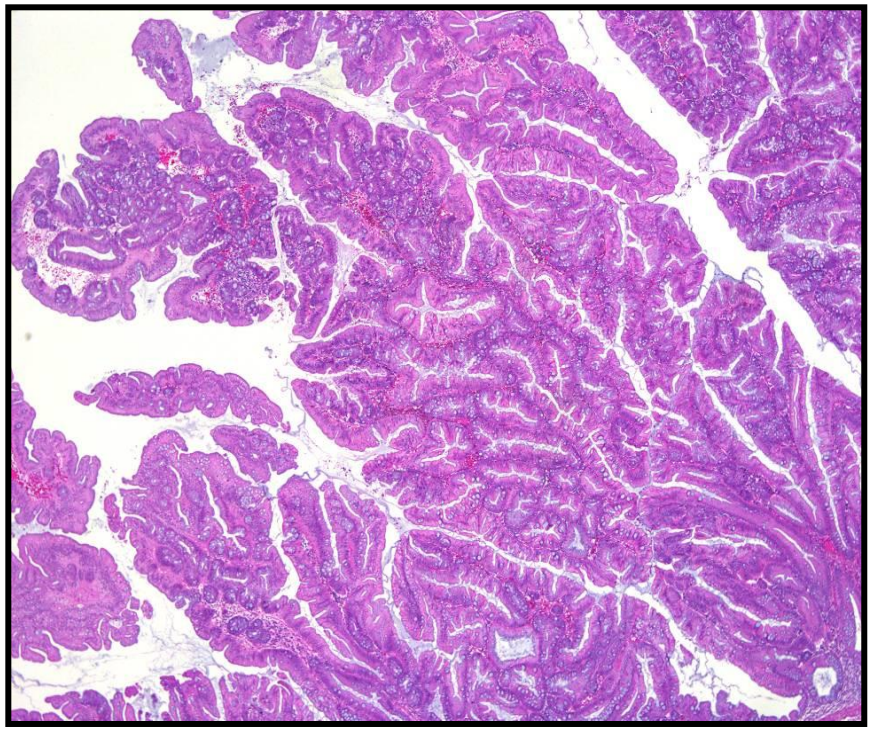

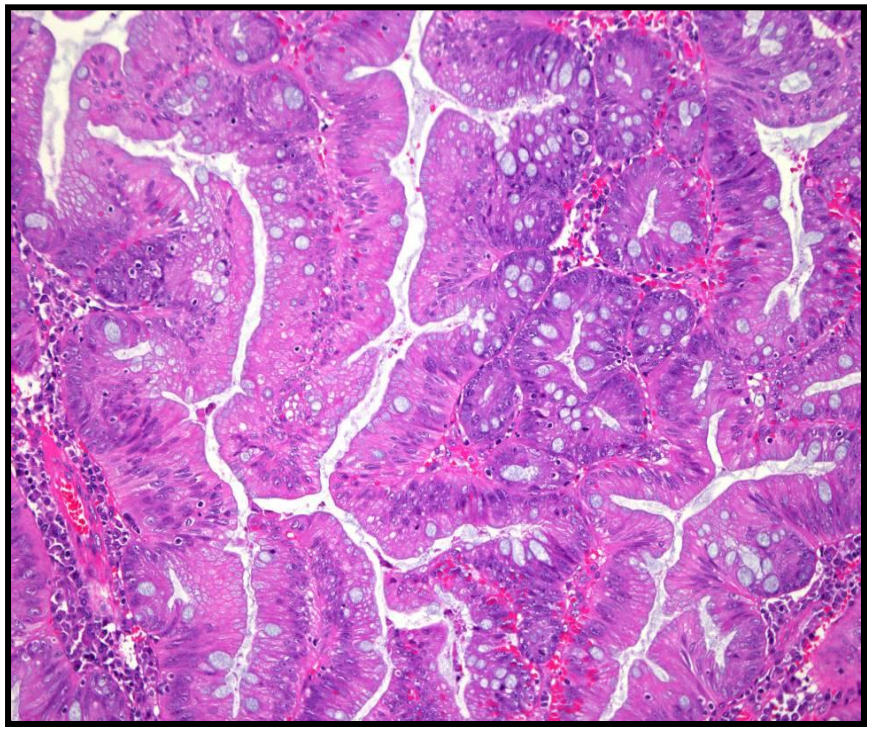

Complex villiform growth pattern with prominent serration

Diffuse eosinophilic cytoplasm

Traditional Serrated Adenoma (TSA)

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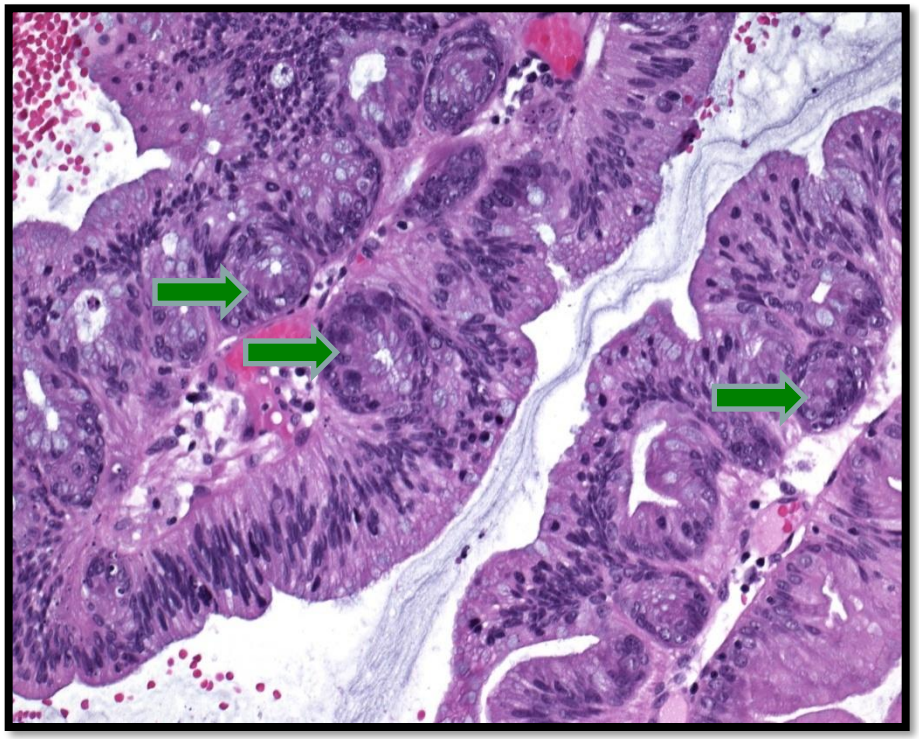

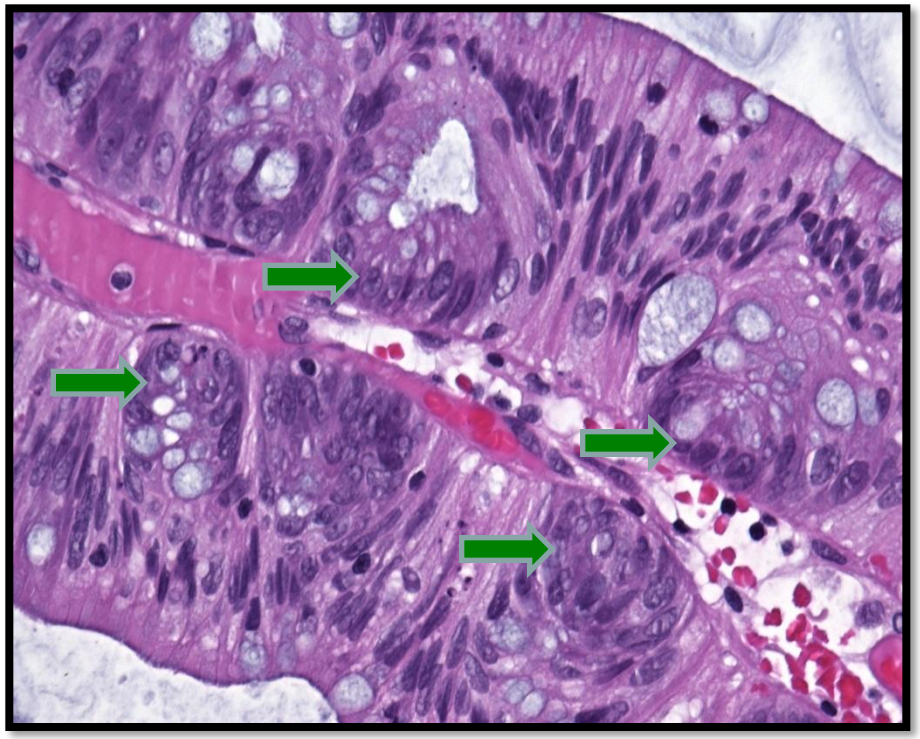

Cytological dysplasia (intraepthelial neoplasia)

Ectopic crypt foci (ECF; arrows)

Hyperplastic Polyposis (Serrated Polyposis According to WHO 2010)

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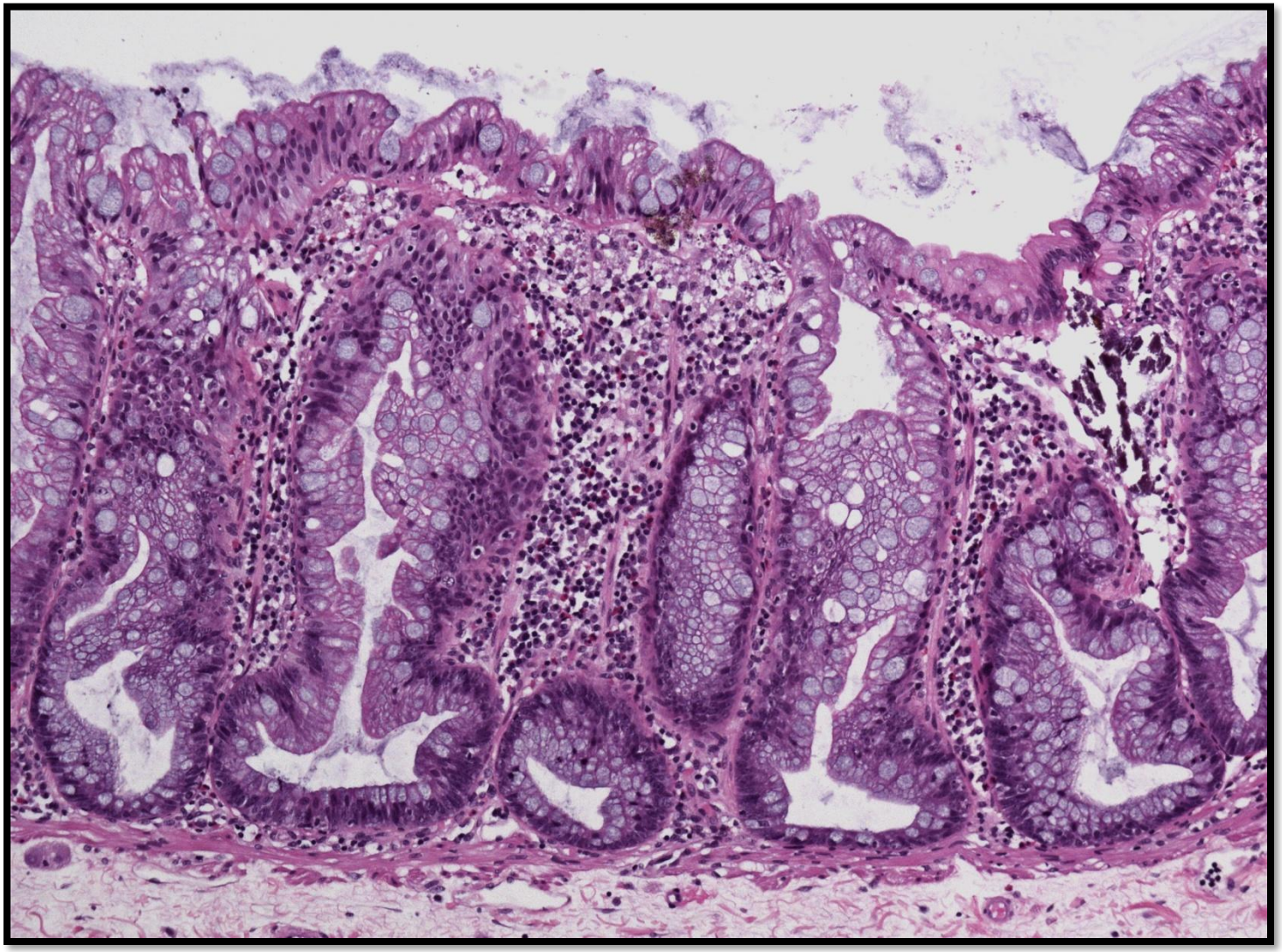
- **Diagnostic Criteria**
  - At least 5 serrated polyps proximal to the sigmoid colon with two or more of these being > 10 mm
  - Any number of serrated polyps proximal to the sigmoid colon in an individual who has a first-degree relative with hyperplastic (serrated) polyposis
  - More than 20 serrated polyps of any size, but distributed throughout the colon
- **Variants**
  - **Type 1:** Multiple lesions (larger and often more proximal) → substantial cancer risk
  - **Type 2:** Numerous small (< 5 mm) lesions distributed throughout the colon → modest cancer risk

Torlakovic und Snover. Gastroenterology 1996
Snover et al. WHO Classification 2010

Hyperplastic Polyp?



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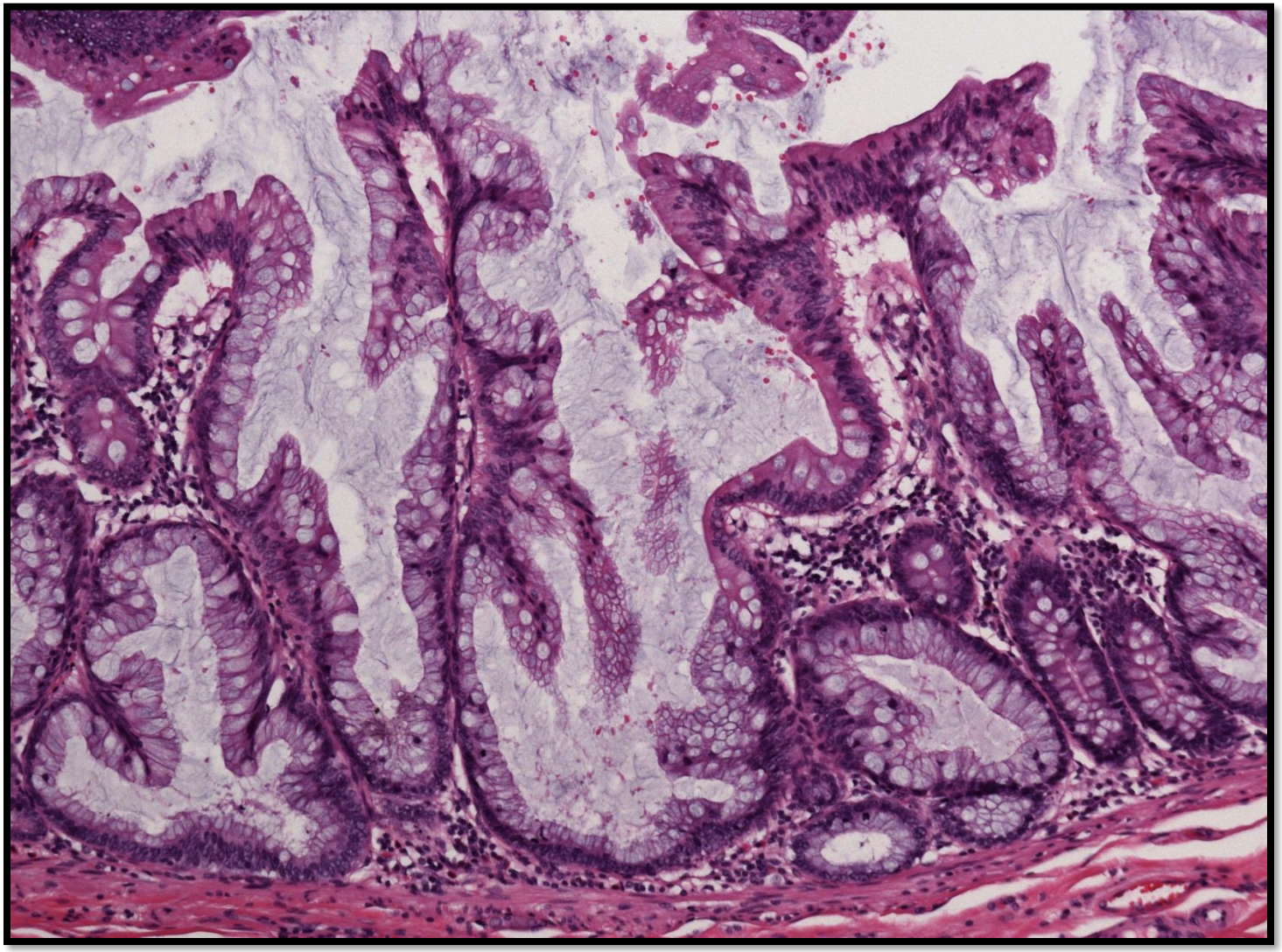
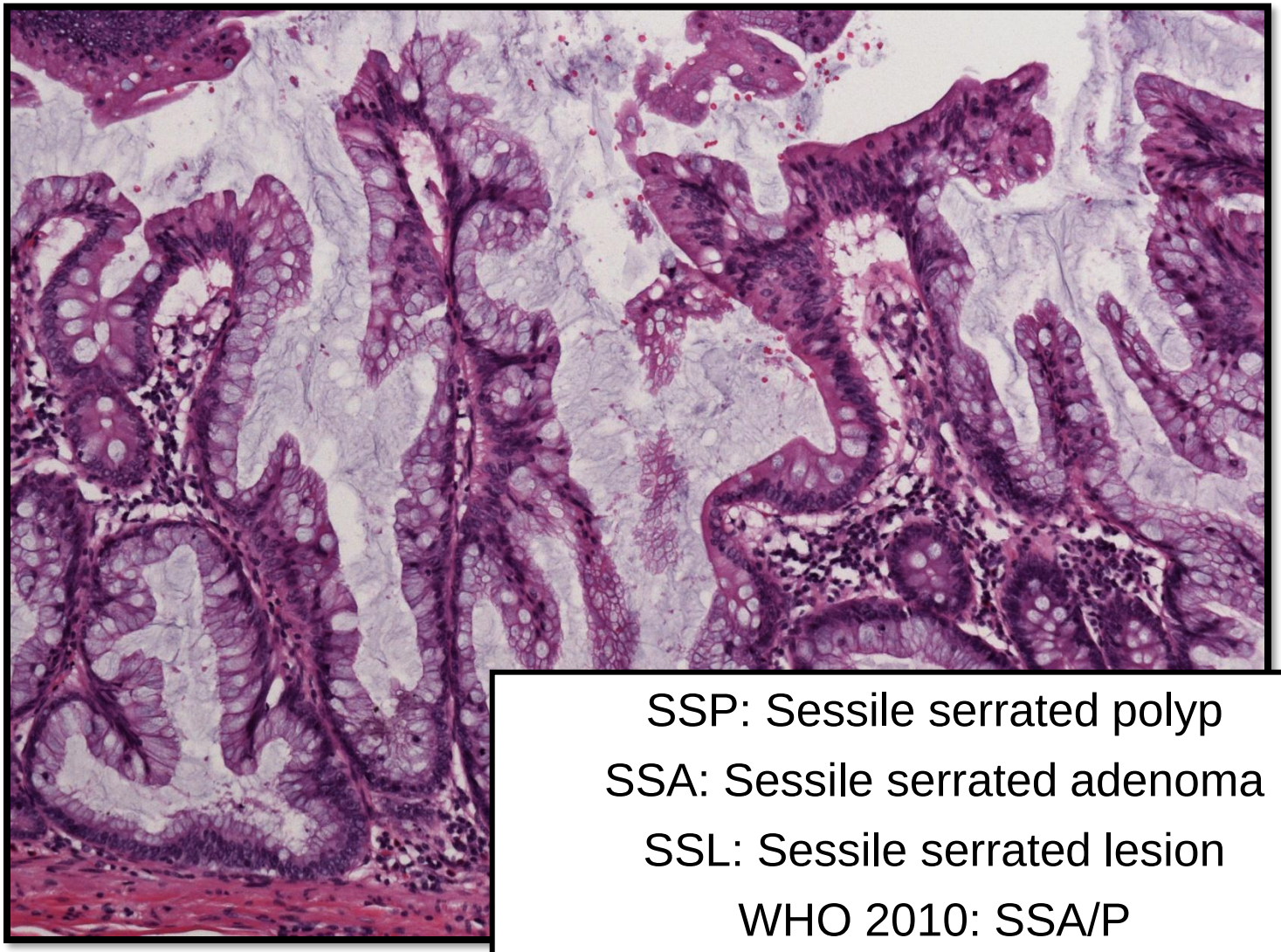
Hyperplastic Polyp?



Hyperplastic Polyp?



SSP: Sessile serrated polyp

SSA: Sessile serrated adenoma

SSL: Sessile serrated lesion

WHO 2010: SSA/P

Sessile Serrated Adenoma (SSA/P) / Part I

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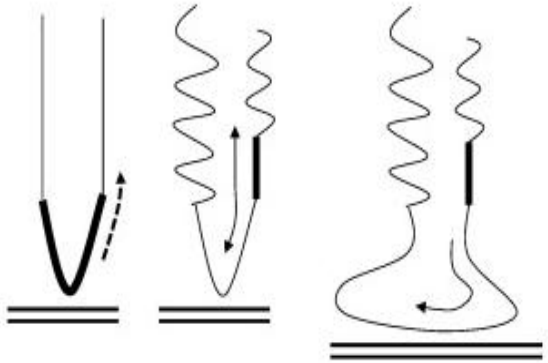
- **Incidence**
  - 15-25% of serrated polyps
- **Morphology**
  - Location: right > left (coecum to transverse colon)
  - Macroscopy / Endoscopy: sessile (non-polypoid) lesion (> 5 mm)
  - **Histology:**
    - (Hyper)Serration in the lower third of the crypts (with and without branching)
    - T- and L-shaped crypts above the muscularis mucosae
    - Inverted crypts (pseudoinvasion) below the muscularis mucosae
    - Columnar dilatation in the lower third of the crypts (with or without mucus → “mucus cap”)
    - Bland cytology (no classical dysplasia)

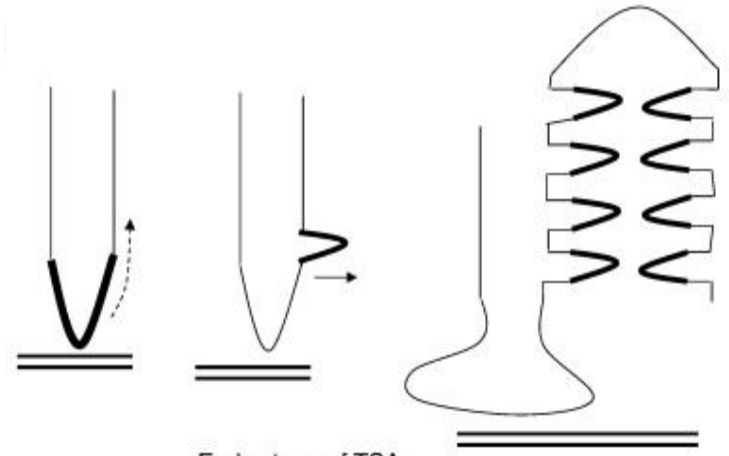
Aust & Baretton. Virchows Arch 2010
Snover. Hum Pathol 2011
Rex et al. Am J Gastroenterol 2012

Morphogenesis of Serrated Crypts



Early stage of SSA/P with movement of proliferative zone to side of crypt (dotted arrow) and bidirectional maturation (solid arrow)

Progression of SSA/P with downward growth of mature epithelium leading to distorted crypt

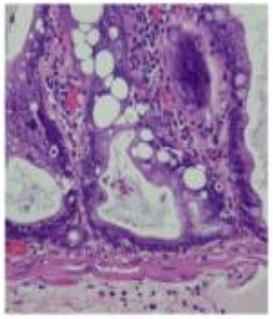


Normal crypt

Early stage of TSA with proliferative zone on side of crypt. Outward growth creates ectopic crypt (arrow)

Fully developed TSA with multiple ectopic crypts lining villi

Sessile Serrated Adenoma (SSA)



Traditional Serrated Adenoma (TSA)

Snover. Hum Pathol 2011

Sessile Serrated Adenoma (SSA/P) / Part II

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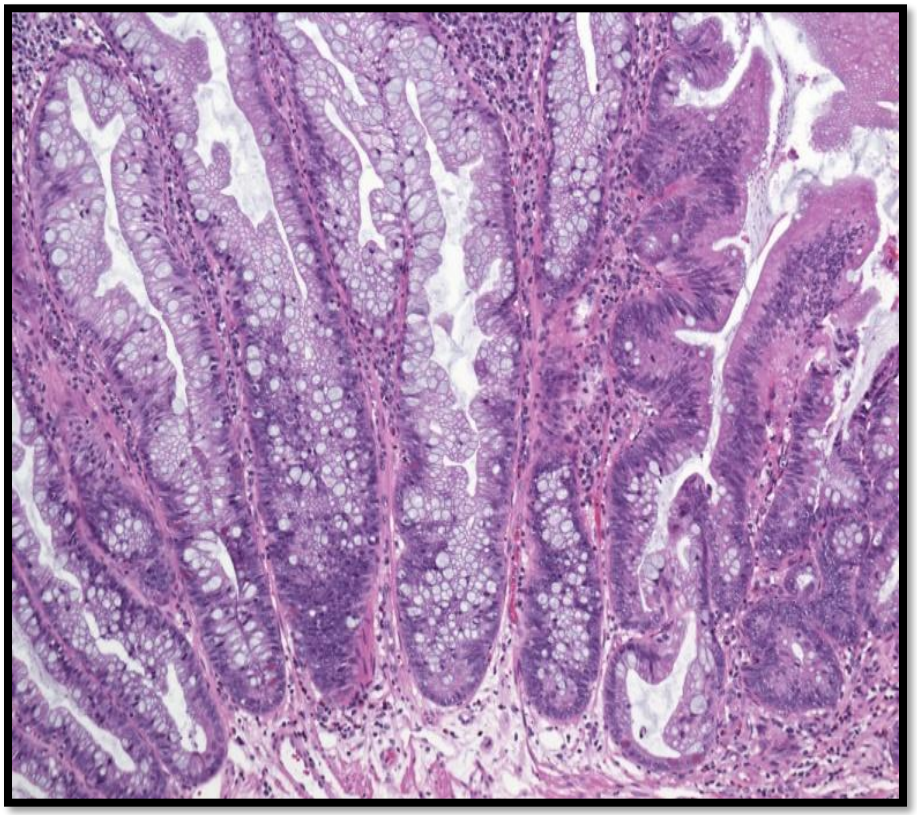
- **Minimum Morphological Criteria (DD HP)**
  - If more than two or three contigious crypts show features of SSA/P the lesion should be classified as SSA/P (WHO 2010)
- **Molecular Pathology**
  - *BRAF* mutation
  - CIMP phenotype
- **Cytological dysplasia is not present in uncomplicated SSA/P but develops with progression towards carcinoma (often in conjunction with methylation of MLH1 and MSI)**
  - SSA/P with dysplasia (formerly „mixed polyp“: SSA with TA, TVA, VA, TSA)
  - SSA/P as indicator of „advanced neoplasia“ and/or cancer in follow-up endoscopies

Li et al. Am J Gastroenterol 2009
Schreiner et al. Gastroenterology 2010
Lu et al. Am J Surg Pathol 2010

SSA/P with Dysplasia



Baretton, Langner et al. Pathologe 2011

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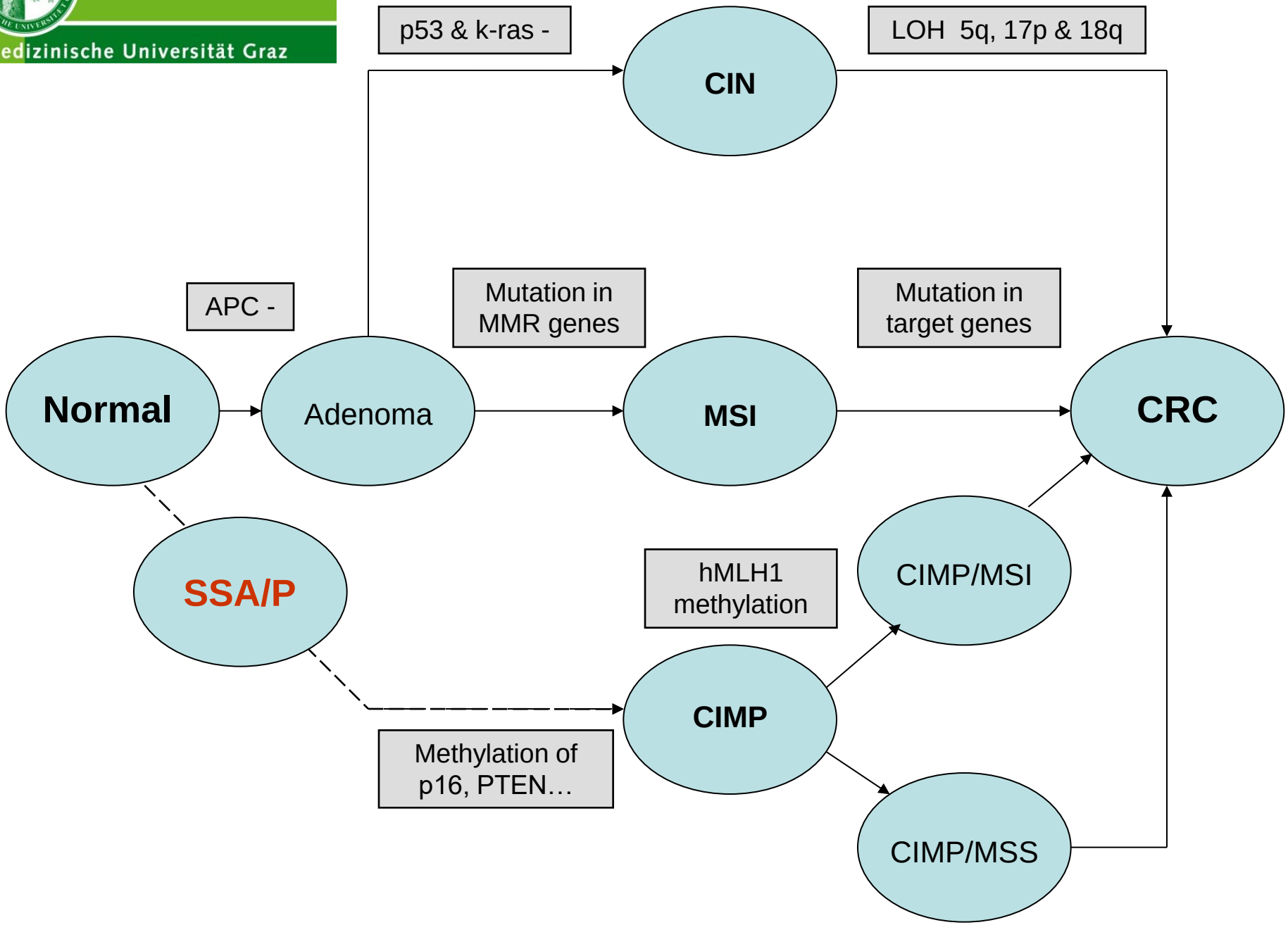

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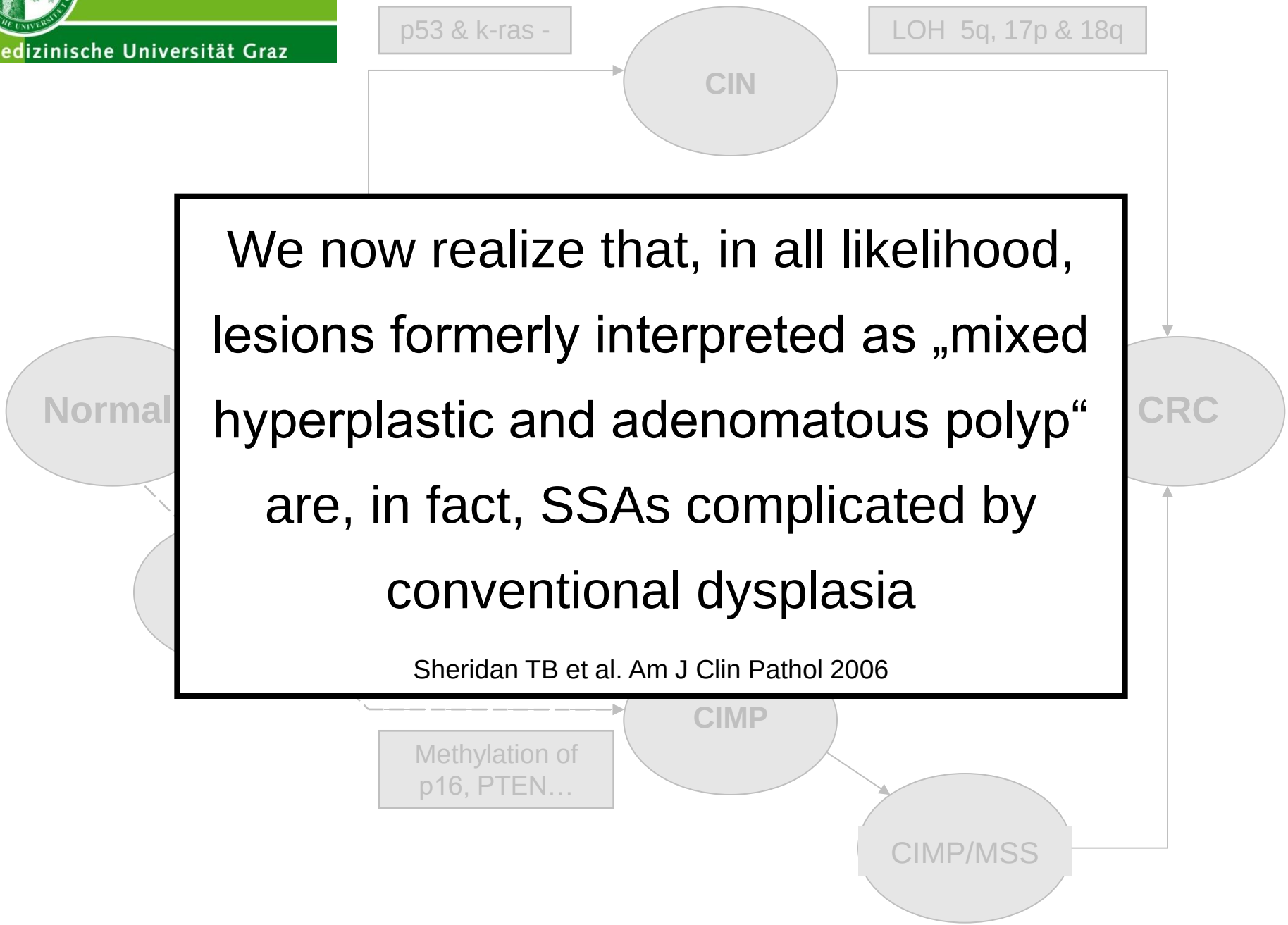

We now realize that, in all likelihood, lesions formerly interpreted as „mixed hyperplastic and adenomatous polyp“ are, in fact, SSAs complicated by conventional dysplasia

Sheridan TB et al. Am J Clin Pathol 2006

High Prevalence of Sessile Serrated Adenomas with *BRAF* Mutation: A Prospective Study of Patients Undergoing Colonoscopy

Spring KJ et al. Gastroenterology 131(11):1400-1407, 2006

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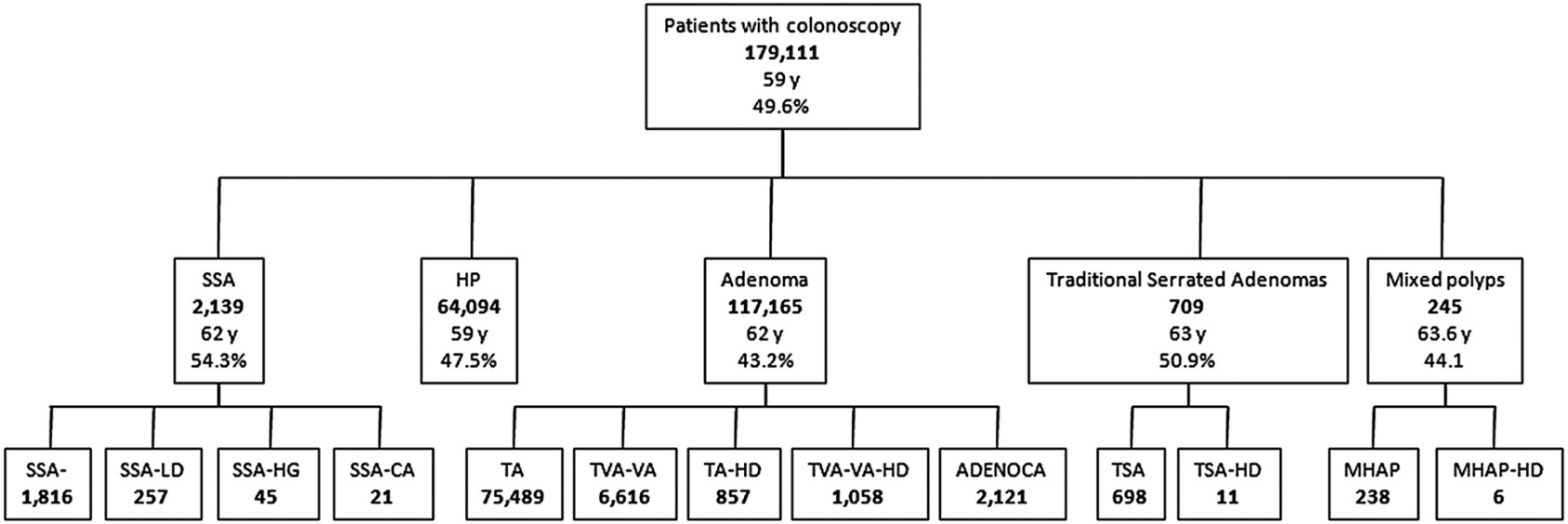
Polyp Type	Number (n=414)	Proximal Location	Distal Location
HPP	120 (29%)	35 (29%)	85 (71%)
SSA	**36 (9%)**	**27 (75%)**	**9 (25%)**
TSA	3 (1%)	2 (66%)	1 (33%)
MP	7 (2%)	4 (57%)	3 (43%)
Tubular Adenoma	237 (57%)	176 (74%)	61 (26%)
Tubulovillous Adenoma	11 (3%)	6 (55%)	5 (45%)

Sessile Serrated Adenomas: prevalence of dysplasia and carcinoma in 2139 patients

Lash RH et al. J Clin Pathol 63(8):681-686, 2010



Right	81%
Left	11%
Right and left	3%
Unknown	4%

SSA without Dysplasia	85%
SSA with LG Dysplasia	12%
SSA with HG Dysplasia	2%
SSA with Carcinoma	1%

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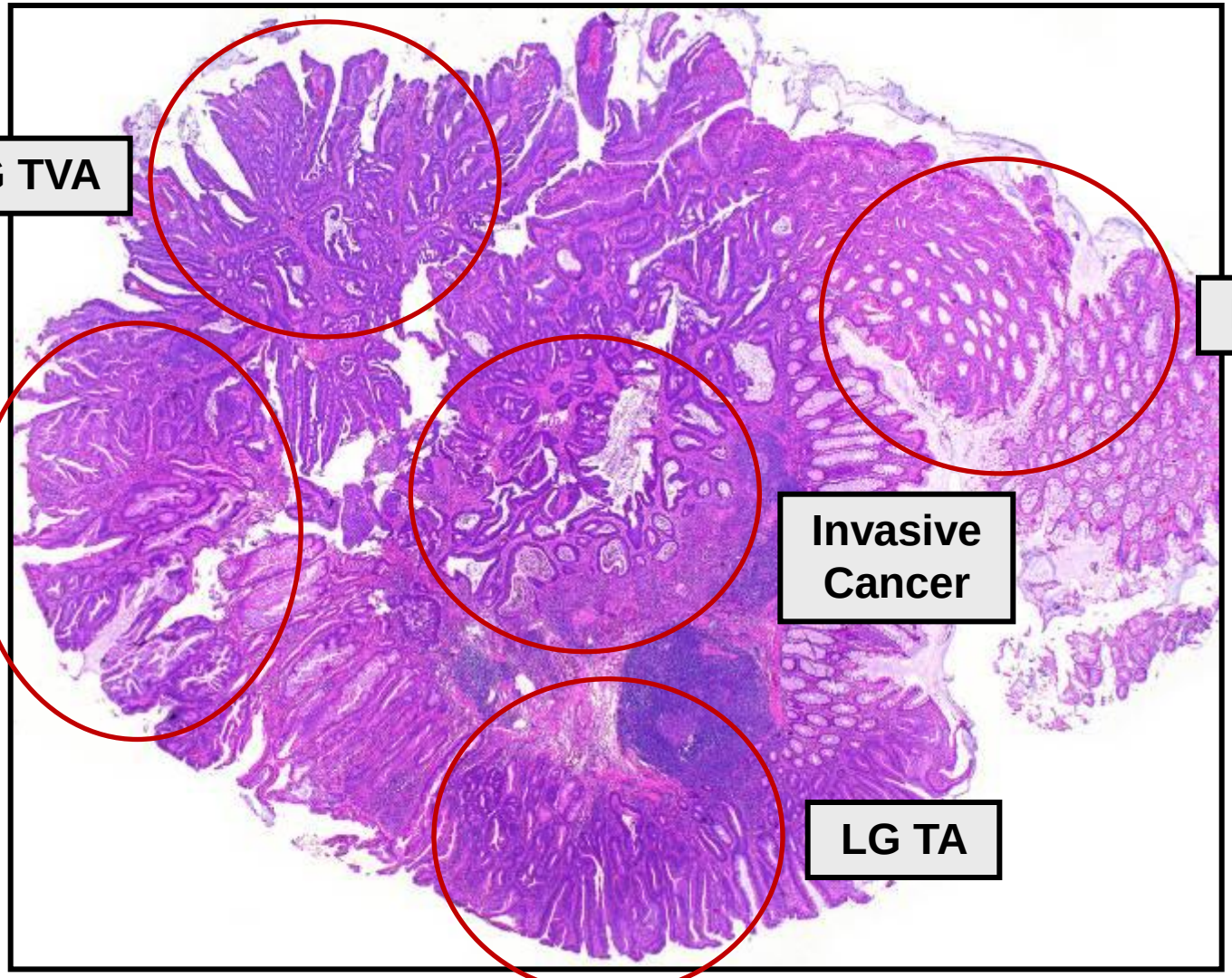
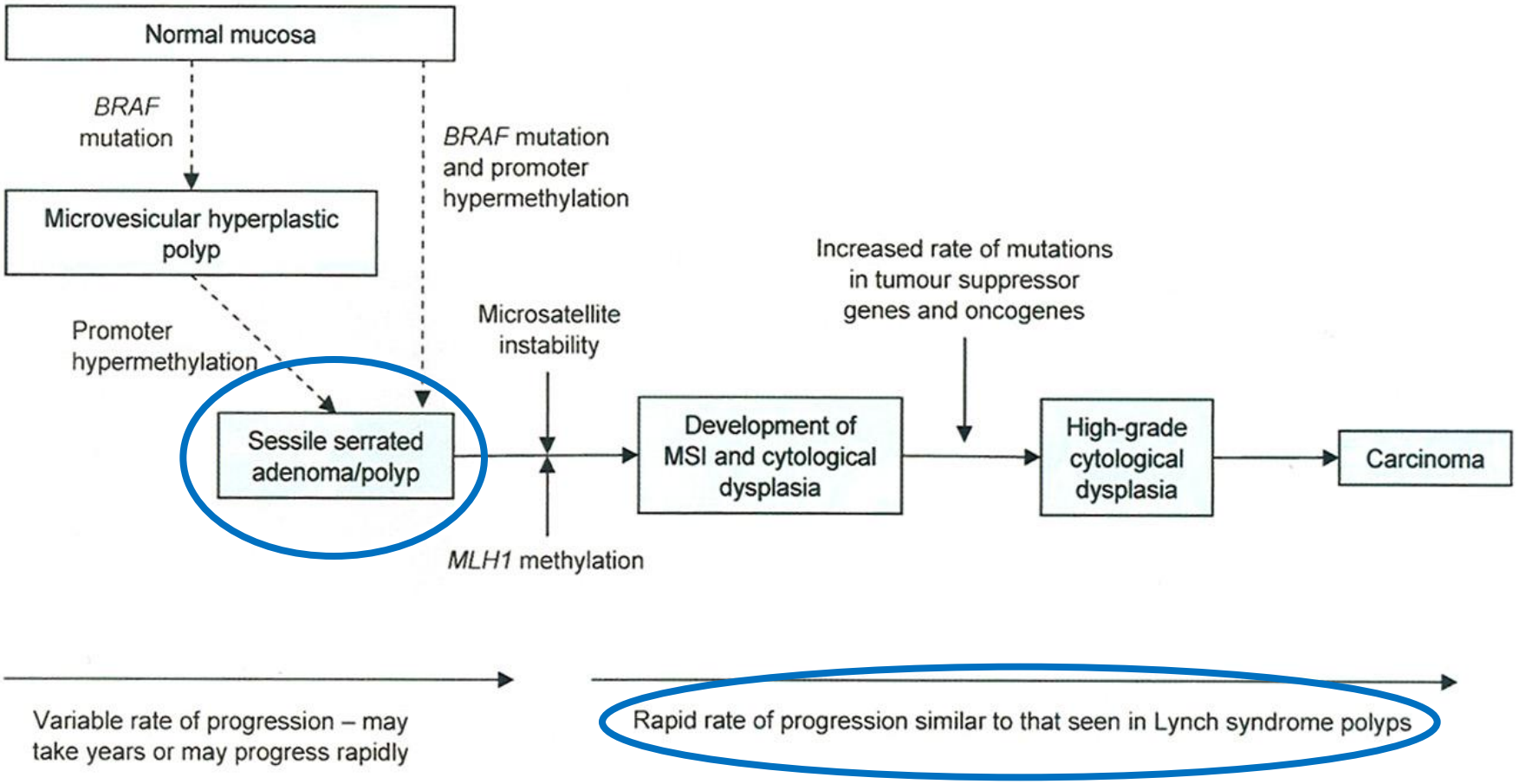
- Can immunohistochemistry help to classify serrated lesions?

Serrated Pathway

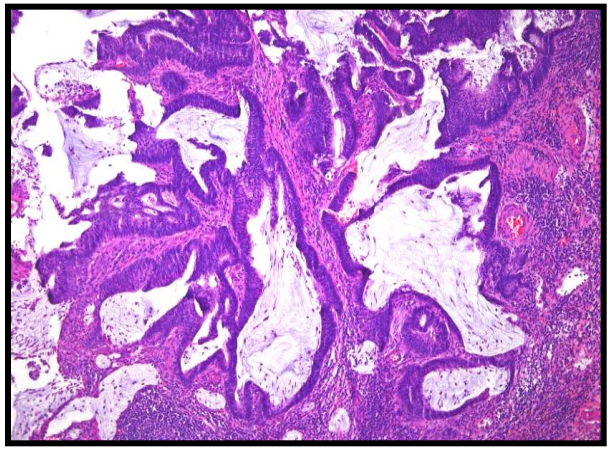


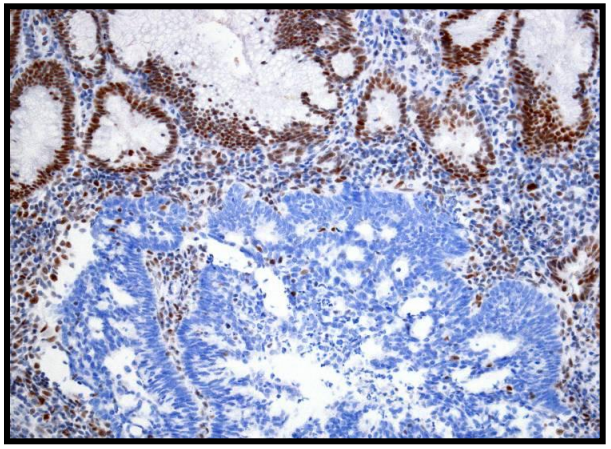
Serrated Route to Cancer

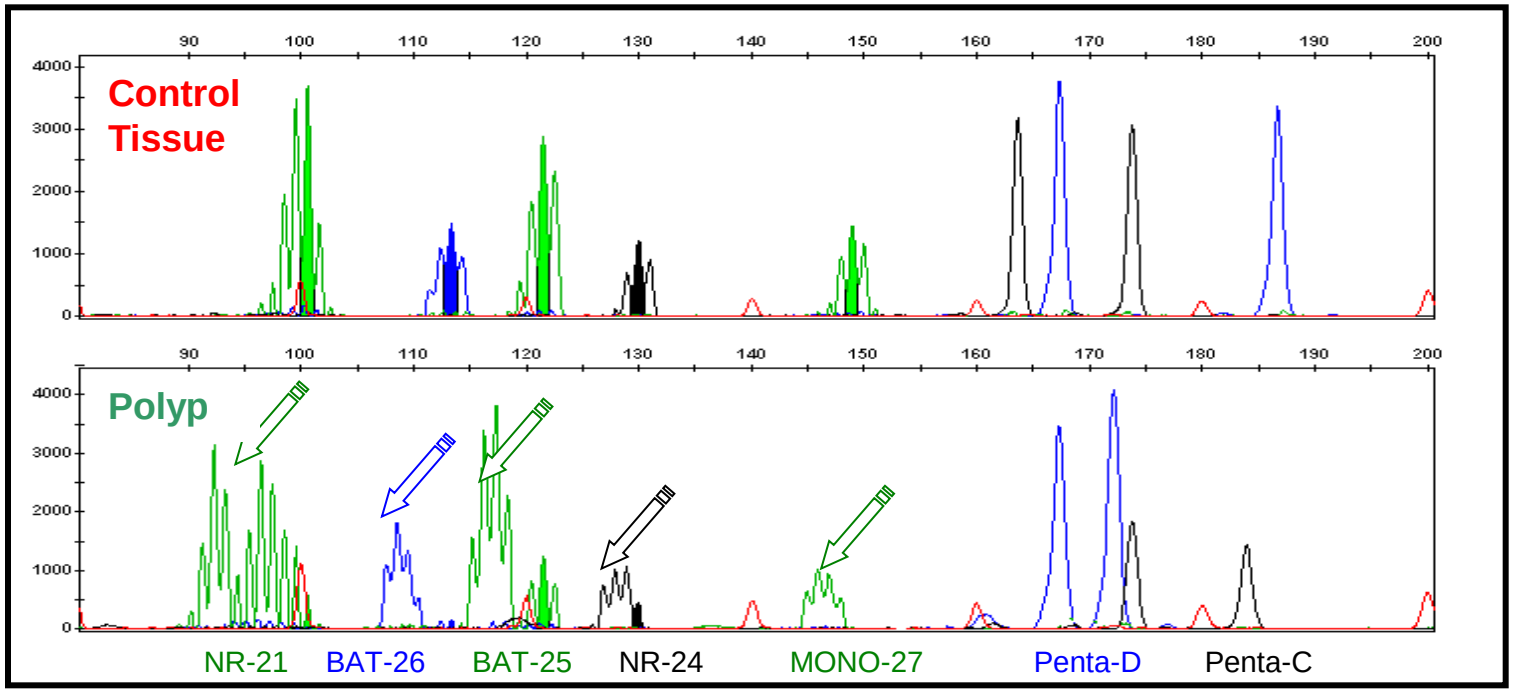


WHO Classification 2010

Serrated Pathway







Dig Dis Sci (2009) 54:906–909
DOI 10.1007/s10620-008-0407-7

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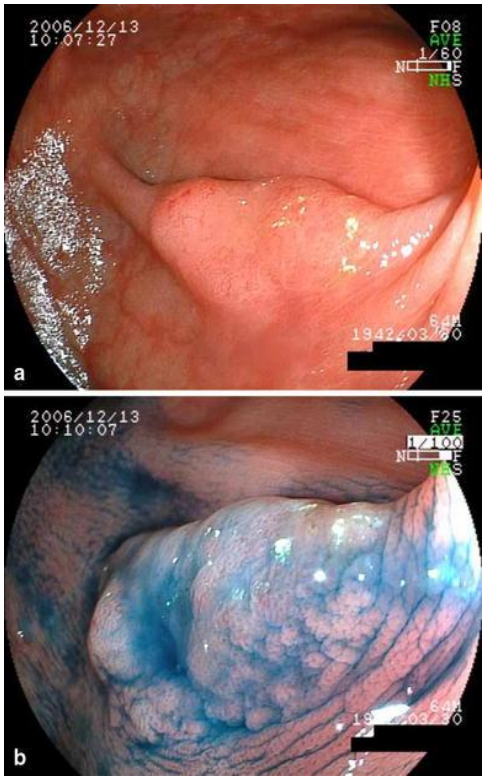
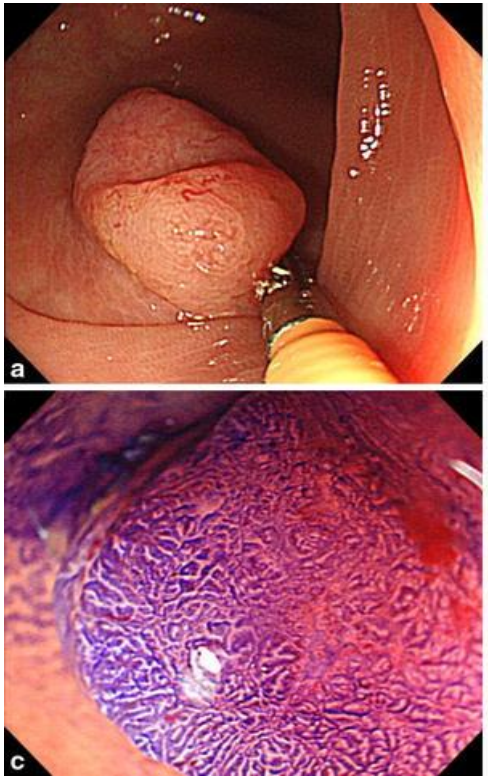
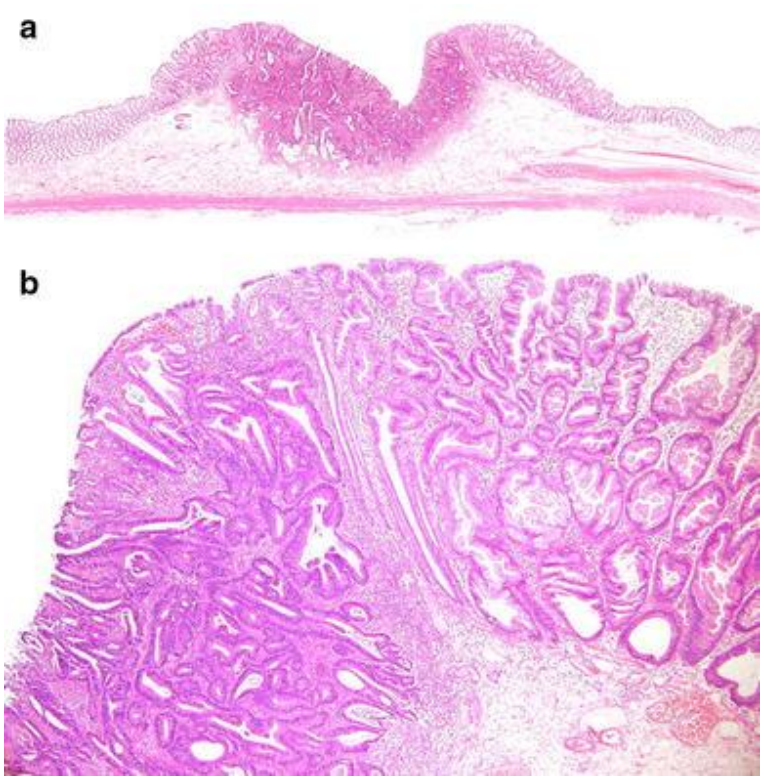
CASE REPORT

Progression of a Sessile Serrated Adenoma to an Early Invasive Cancer Within 8 Months

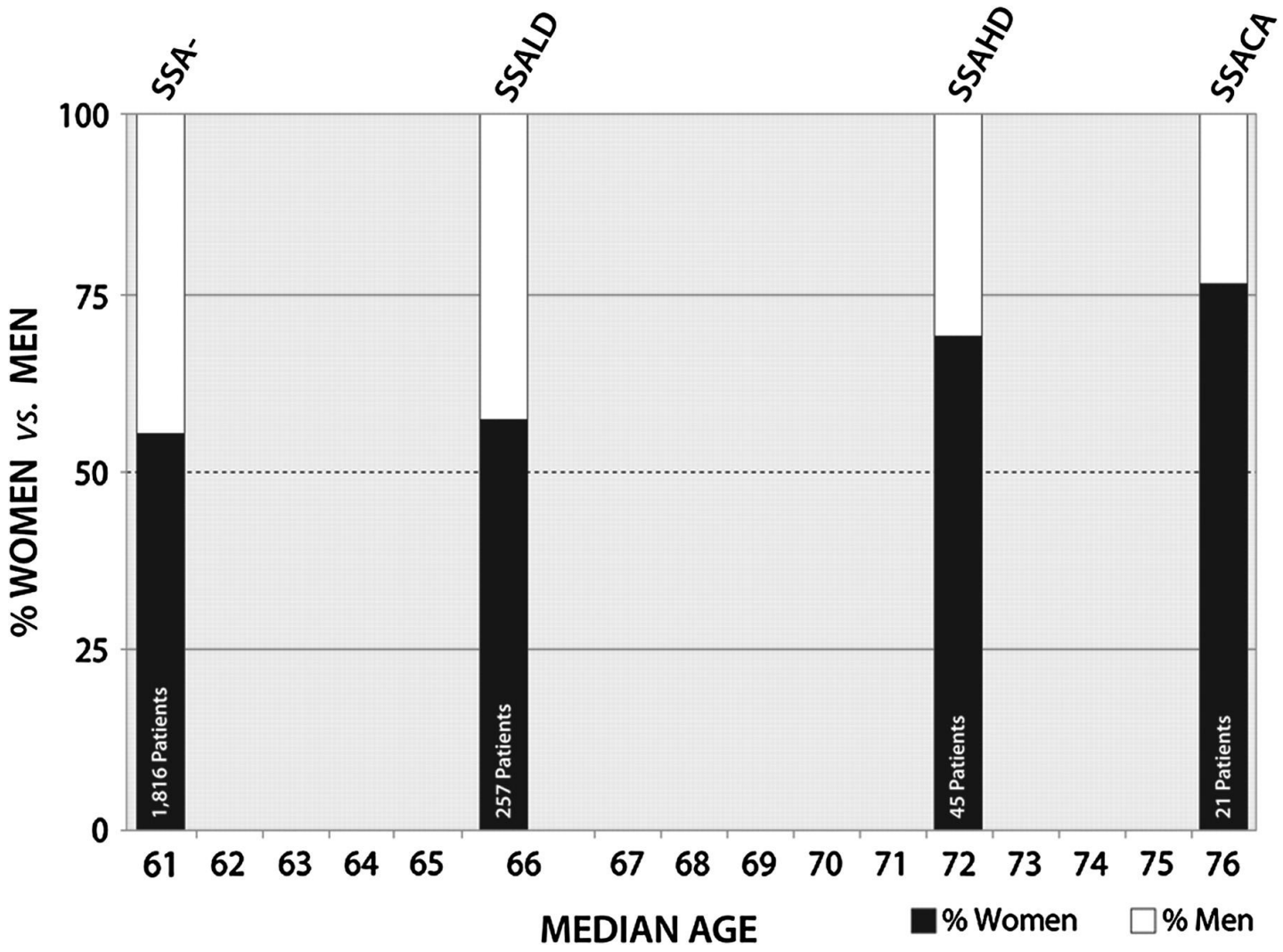
Yasuhiro Oono · Kuangi Fu · Hisashi Nakamura · Yosuke Iriguchi · Akihiko Yamamura · Yasuhiro Tomino · Johji Oda · Masaru Mizutani · Satoshi Takayanagi · Daisuke Kishi · Tomoaki Shinohara · Kozo Yamada · Jun Matumoto · Kazuhiro Imamura





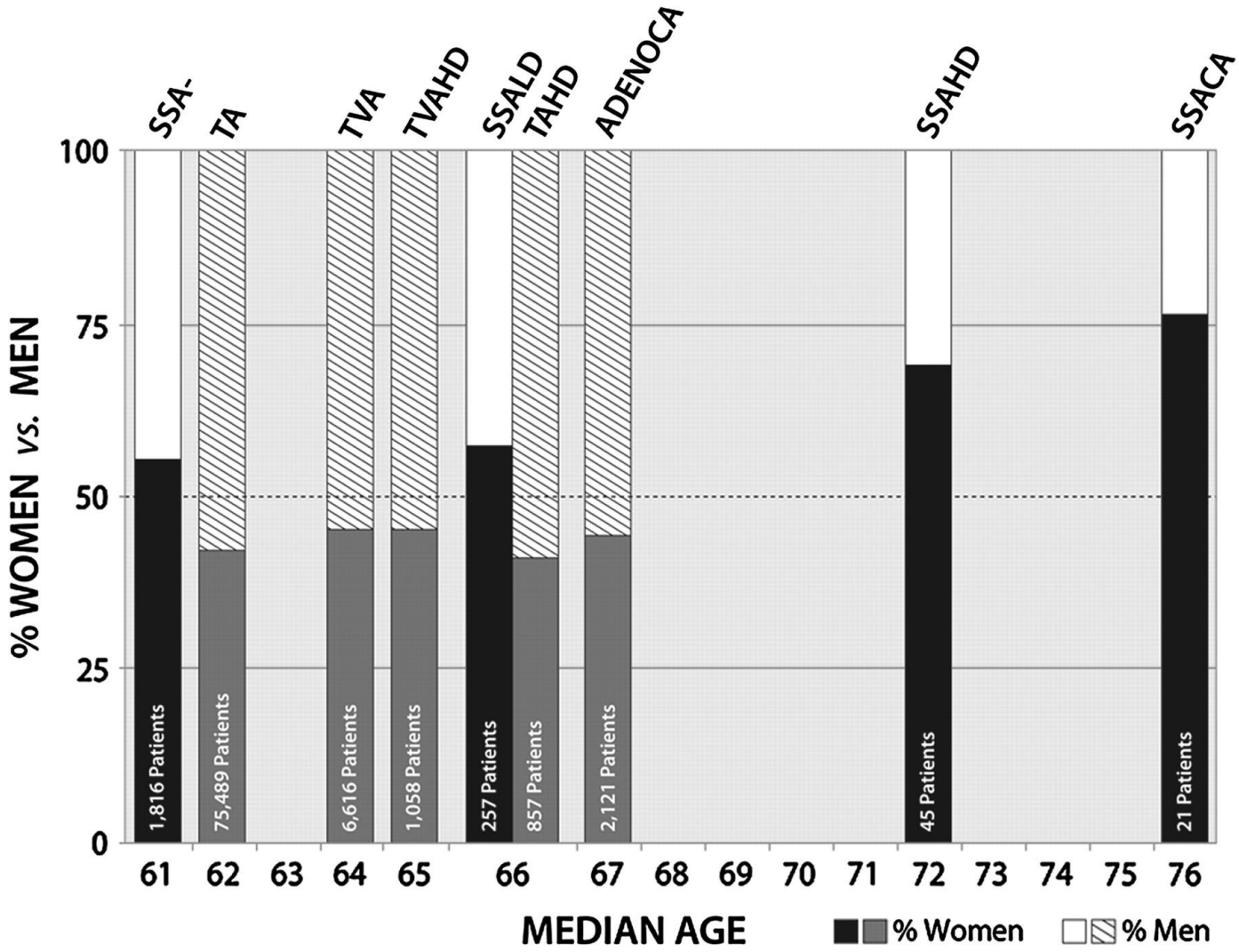


Serrated Pathway
Age at Time of Diagnosis



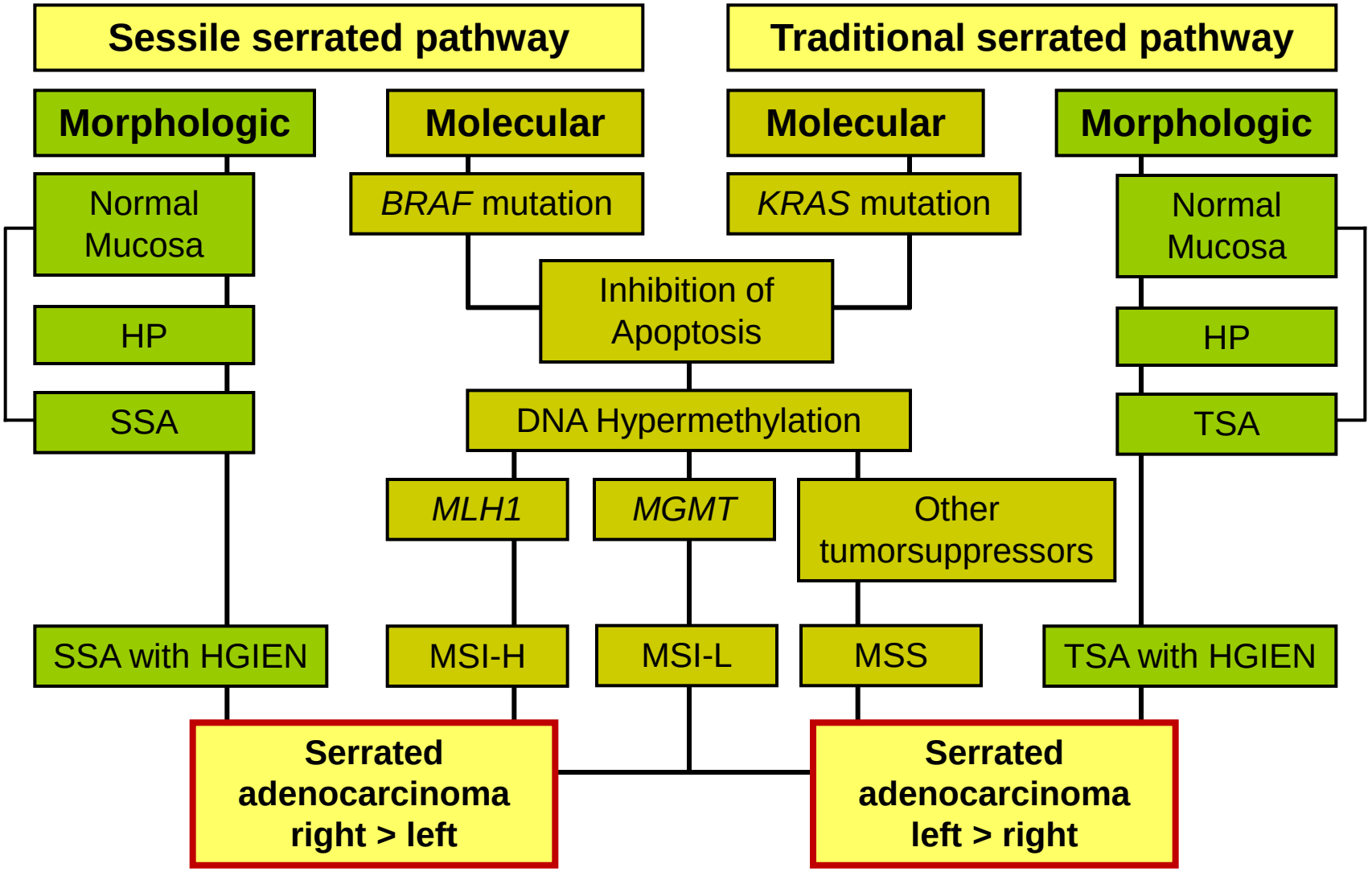
Lash et al. J Clin Pathol 2010

Serrated Pathway
Age at Time of Diagnosis



Lash et al. J Clin Pathol 2010

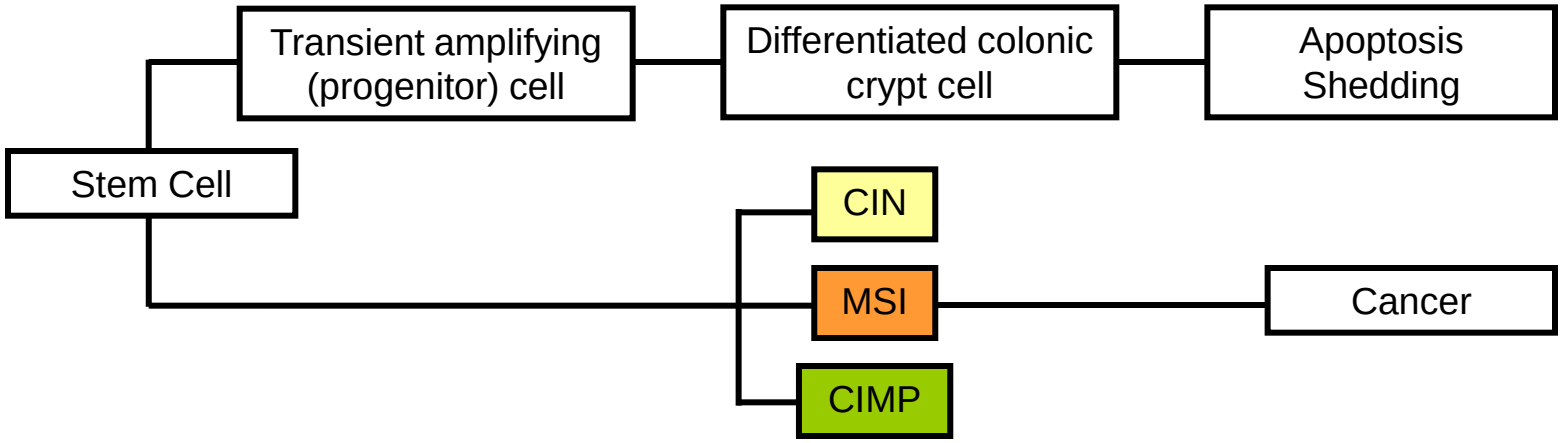
Serrated Adenocarcinoma



Mäkinen, Histopathology 2007
Noffsinger, Annu Rev Pathol Mech Dis 2009

Colonic Stem Cells: Implications for Carcinogenesis

Medizinische Universität Graz

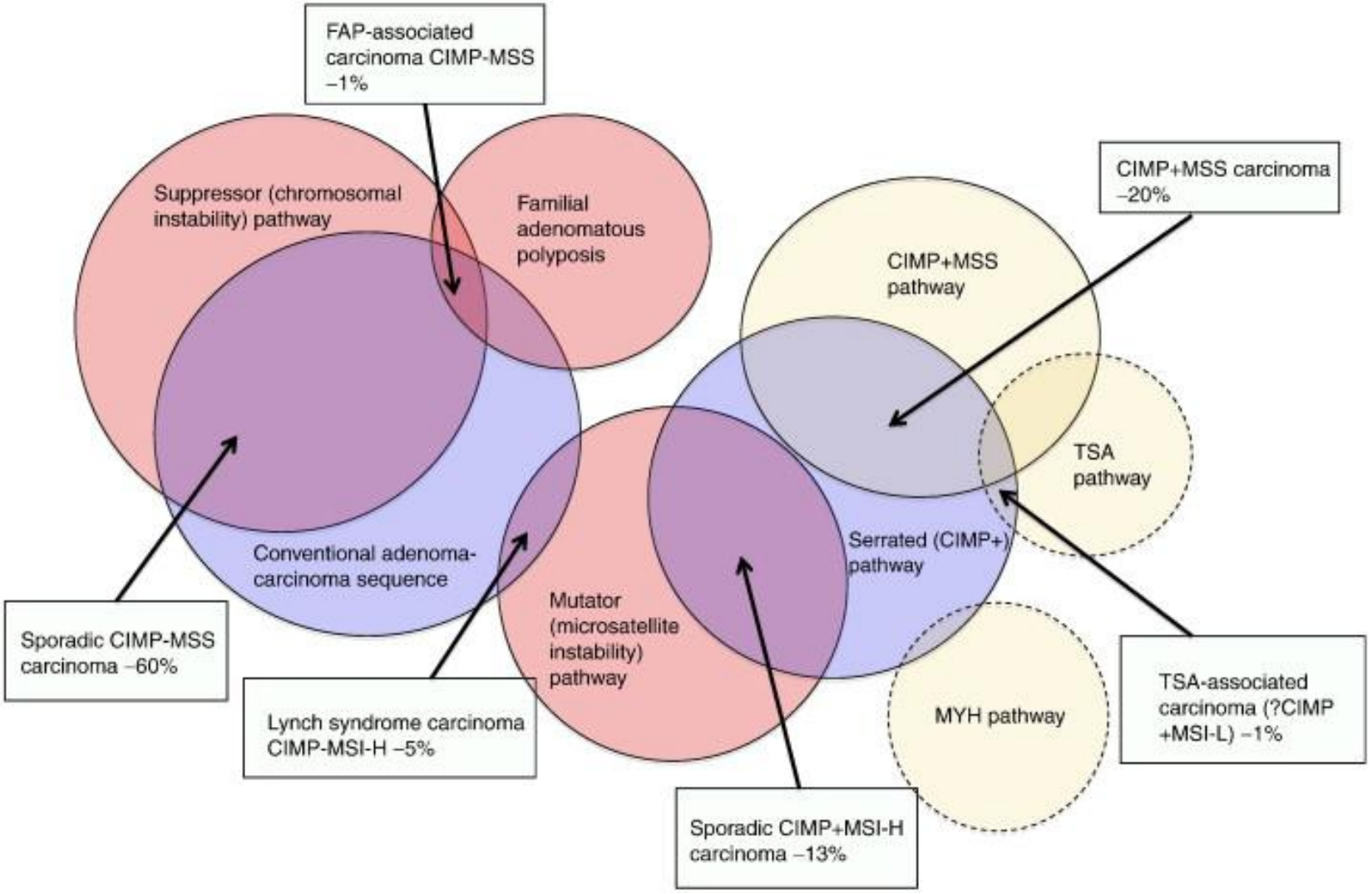


Type 1 (MSI-H)	**SSA/P (HP)** CpG-Methylation of MLH1 / Mut. *BRAF* / R > L	**12%**
Type 2 (MSI-L, MSS)	**SSA/P (HP)** CpG-Methylation of MGMT / Mut. *BRAF* / R > L	**8%**
Type 3 (MSI-L, MSS)	**TSA or classical adenoma** CpG-Methylation of MGMT / Mut. *KRAS, p53* / L > R	**20%**
Type 4 (MSS)	**Classical adenoma** Mut. *APC, p53* / L > R	**57%**
Type 5 (MSI-H, HNPCC)	**Classical adenoma** Mut. MMR-genes / R > L	**3%**

Jass, Histopathology 2007 and Holinski-Feder & Morak, J Gastroenterol Hepatol Erkr 2010

Molecular Mechanisms of CRC Developed via the Serrated Pathway

Medizinische Universität Graz



Snover. Hum Pathol 2011

Outline

Medizinische Universität Graz

- Pathogenesis of colorectal cancer: adenoma-carcinoma-sequence
- Colorectal polyps
  - Hyperplastic polyp (HP)
  - Traditional serrated adenoma (TSA)
  - Sessile serrated adenoma (SSA)
  - Classical adenoma (TA, TVA, VA)
- The serrated route: implications for the classification of colorectal cancer
- **Can immunohistochemistry help to classify serrated lesions?**

Mucin Apoproteins in Colorectal Polyps

Medizinische Universität Graz

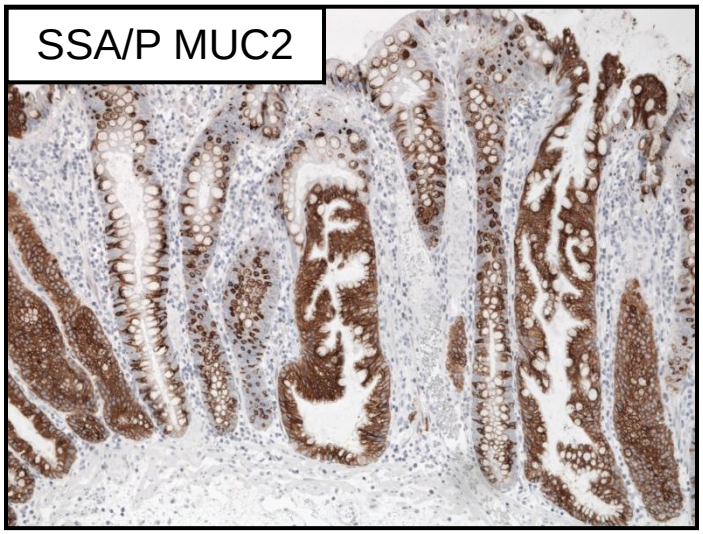
	n	MUC2	MUC5AC	MUC6
Normal large bowel mucosa	10	100% (3/3-3)	40% (0/0-1)	0% (0/0-0)
Tubular Adenoma	16	100% (3/3-3)	69% (1/0-1)	6% (0/0-0)
Hyperplastic Polyp	**15**	**100% (3/3-3)**	**100% (2/2-3)**	**27% (0/0-0,75)**
SSA/P	**29**	**100% (3/3-3)**	**100% (3/2-3)**	**76% (0/0,75-1)**
TSA	12	100% (3/3-3)	100% (1/0-1)	0% (0/0-0)

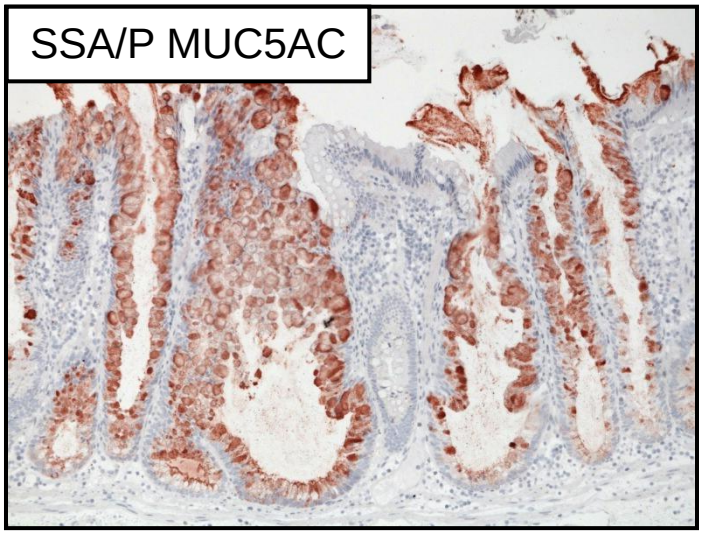
Score 1: <33%, Score 2; 33-66%, Score 3%

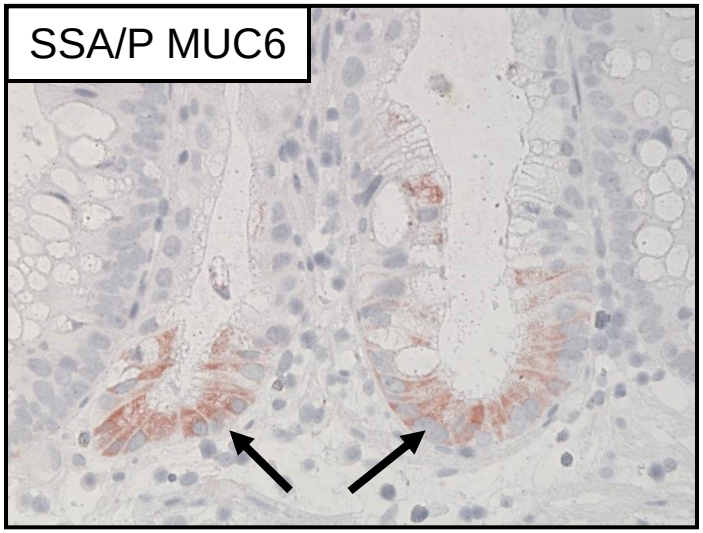
Mochizuka et al. Histochem Cell Biol 2007

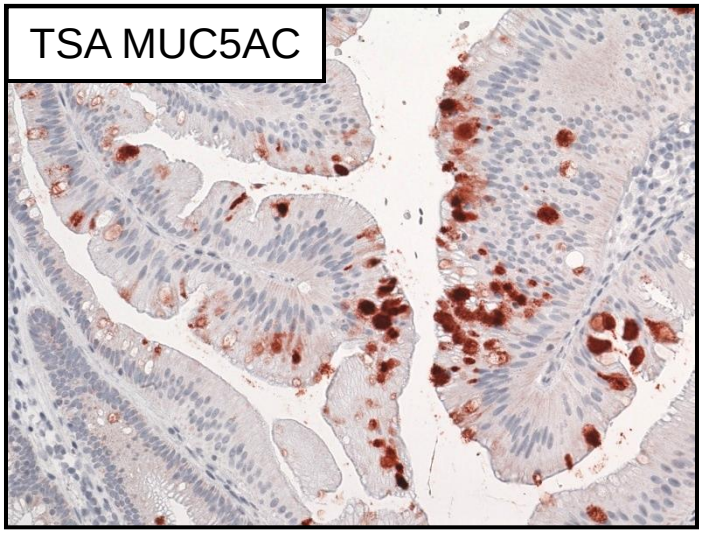
Mucin Apoproteins in Colorectal Polyps

Medizinische Universität Graz





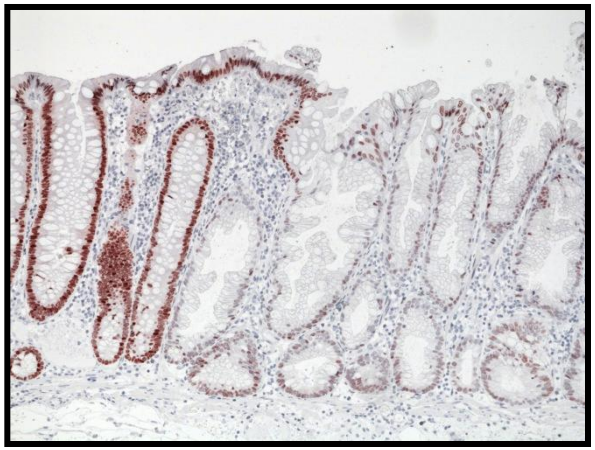


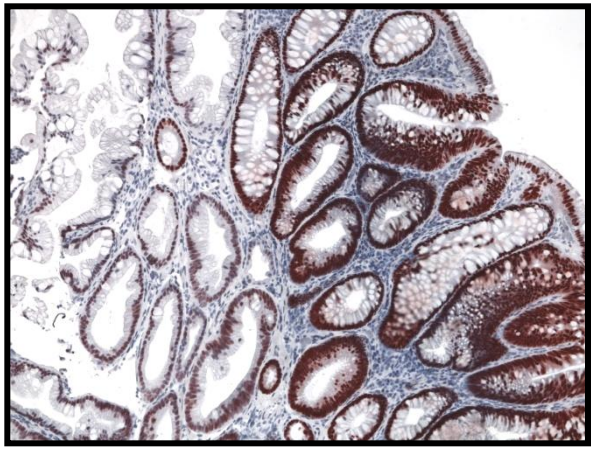


CDX-2 in Normal Mucosa and in Colorectal Polyps

Medizinische Universität Graz

	n	**CDX-2**
Normal large bowel mucosa	10	100% (3/3-3)
Tubular Adenoma	16	100% (3/3-3)
Hyperplastic Polyp	15	100% (2/1-3)
SSA/P	**29**	**100% (1/1-2)**
TSA	12	100% (3/3-3)





Mochizuka et al. Histochem Cell Biol 2007
Wu et al. Am J Clin Pathol 2008
Dhir et al. Int J Cancer 2011

Differential Diagnosis of Colorectal Polyps

Medizinische Universität Graz

	HP *vs* SSA/P	HP *vs* TSA	SSA *vs* TSA
MUC2	**NS**	NS	NS
MUC5AC	**NS**	P<0.01	P<0.01
MUC6	**P<0.05**	NS	P<0.01
CDX-2	**NS**	P<0.01	P<0.01

HP = hyperplastic polyp, SSA/P = sessile serrated adenoma/polyp, TSA = traditional serrated adenoma

Mochizuka et al. Histochem Cell Biol 2007

Mucin apoproteins in colorectal polyps

Medizinische Universität Graz

	n	MUC2	MUC5AC	MUC6
Hyperplastic Polyp	65	100%	75%	17%
SSA/P	51	100%	80%	39%
TSA	72	100%	43%	4%
P-Value (HP *vs* SSA/P)		**1.0**	**0.65**	**0.01**
P-Value (SSA/P *vs* TSA)		1.0	<0.001	<0.001
P-Value (TSA *vs* HP)		1.0	<0.001	0.02

Fujita et al. Virchows Archiv 2010

The value of MUC6 in differential diagnosis

Medizinische Universität Graz

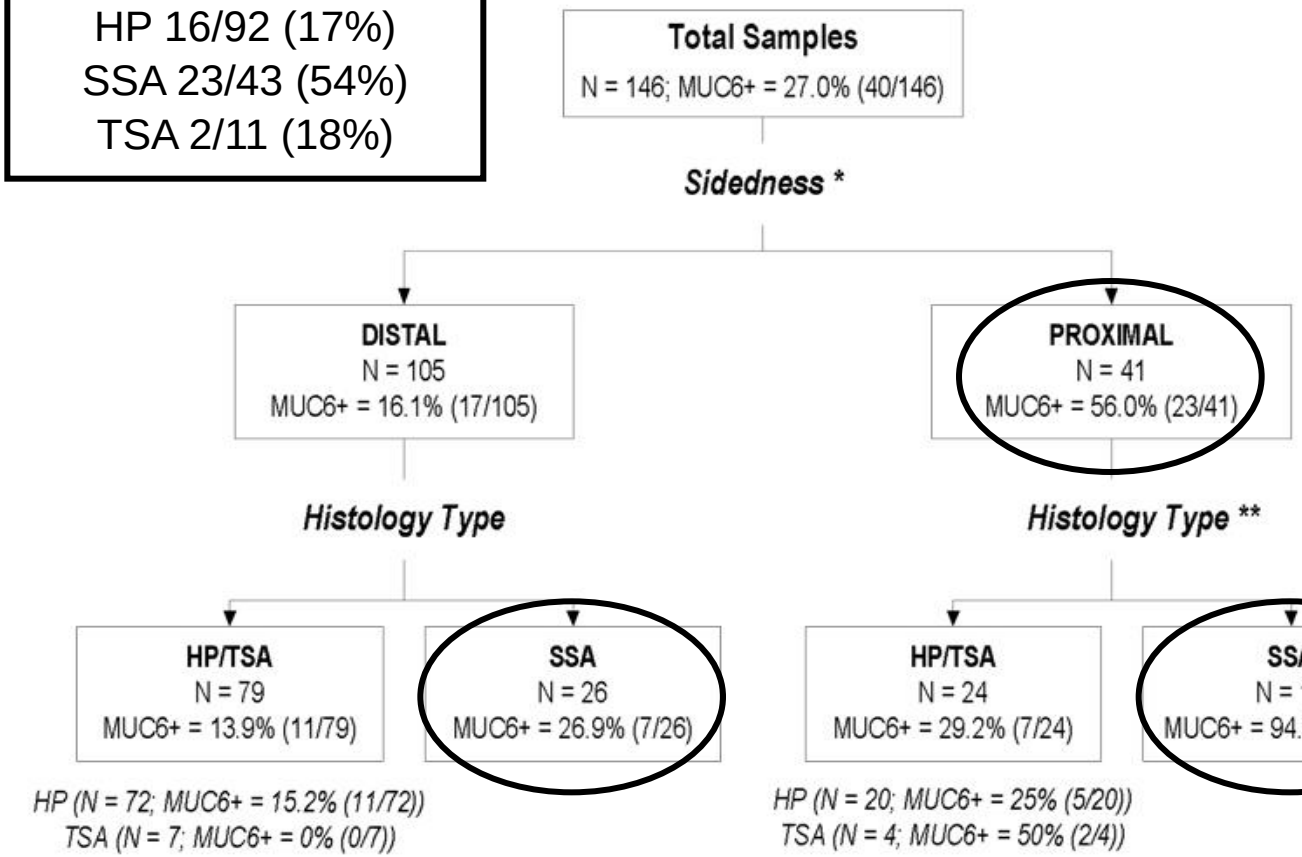
	HP (n=48)	SSA/P (n=26)	SSA/P with dysplasia (n=7)	TSA (n=13)
Location (right *vs* left)	1 : 47	21 : 5	6 : 0 (unknown in one case)	0 : 13
Size	2-13 (5 mm)	3-18 (8 mm)	4-12 (8 mm)	2-13 (7 mm)
MUC6	**0 (0%)**	**26 (100%)**	**7 (100%)**	**0 (0%)**

Owens et al. Mod Path 2008

The value of MUC6 in differential diagnosis

Medizinische Universität Graz

HP 16/92 (17%)
SSA 23/43 (54%)
TSA 2/11 (18%)



Proximal *vs* distal OR 5.79 (p<0.001)

TSA *vs* HP OR 1.06 (p=0.95)

SSA *vs* HP OR 5.18 (p=0.001)

SSA *vs* TSA OR 4.76 (p=0.09)

MUC6: Specificity for the diagnosis of SSA 82%, sensitivity 54%

Bartley et al. Mod Path 2010

The value of MUC6 in differential diagnosis

Medizinische Universität Graz

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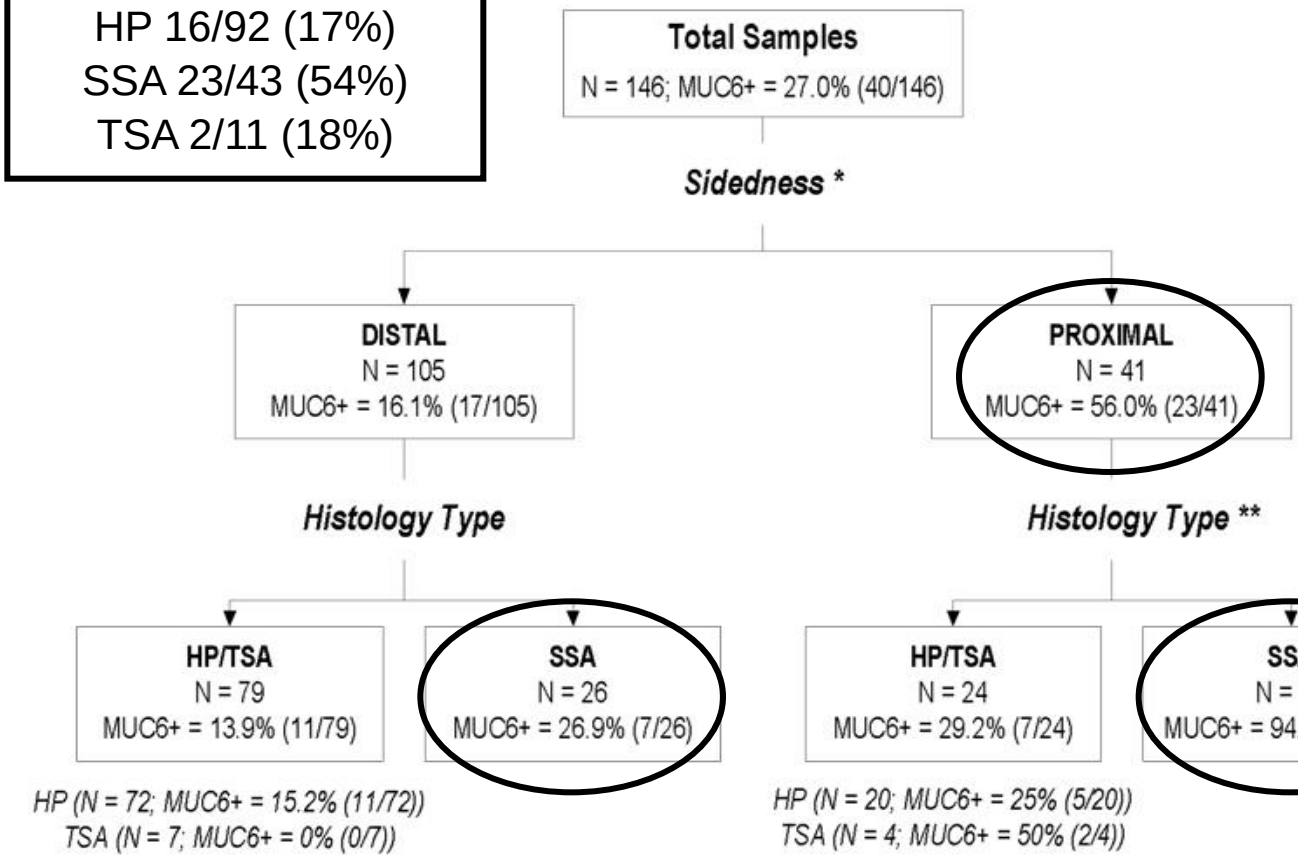

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SSA *vs* TSA OR 4.76 (p=0.09)

MUC6: Specificity for the diagnosis of SSA 82%, sensitivity 54%

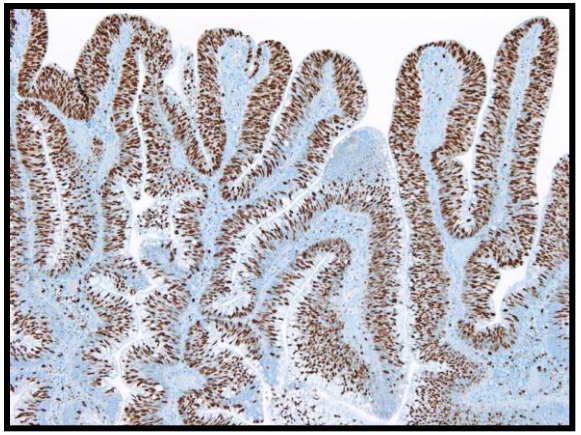
„We conclude that MUC6 is strongly associated with proximal location of serrated polyps, but has only modest utility as a tissue biomarker…“

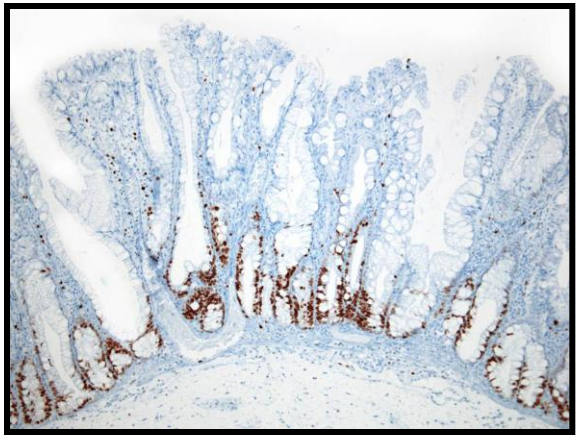
Bartley et al. Mod Path 2010

MIB-1 (Ki67) Proliferation Index of Colorectal Polyps

Medizinische Universität Graz

MIB-1 (Ki-67)	AD	HP	SSA/P	TSA
Upper 1/3	69%	<1%	2%	28%
Middle 1/3	49%	9%	20%	31%
Lower 1/3	30%	60%	65%	38%
Mean Value	50%	23%	29%	32%





Higuchi et al. Histopathology 2005

Value of MLH1 in Differential Diagnosis

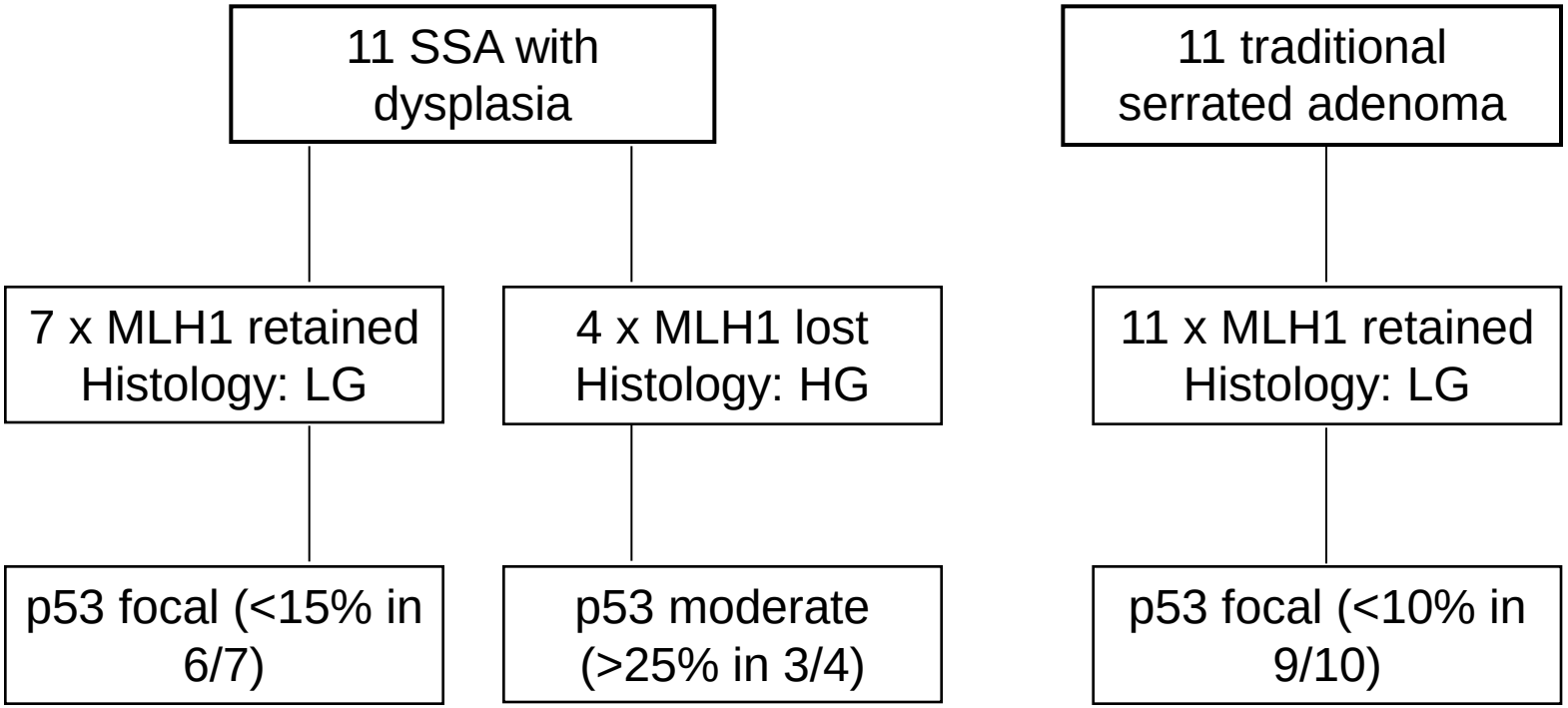
Medizinische Universität Graz

	HP (n=48)	SSA/P (n=26)	SSA/P with Dysplasia (n=7)	TSA (n=13)
Location (right *vs* left)	1 : 47	21 : 5	6 : 0 (unknown in one case)	0 : 13
Size	2-13 (5 mm)	3-18 (8 mm)	4-12 (8 mm)	2-13 (7 mm)
MUC6	0 (0%)	26 (100%)	7 (100%)	0 (0%)
MLH1	**48 (100%)**	**26 (100%)**	**2 (29%)**	**13 (100%)**

Sheridan et al. Am J Clin Pathol 2006
Owens et al. Mod Path 2008

MLH1 and p53 Immuno-histochemistry in the DD SSA/P with Dysplasia (IEN) *vs* TSA

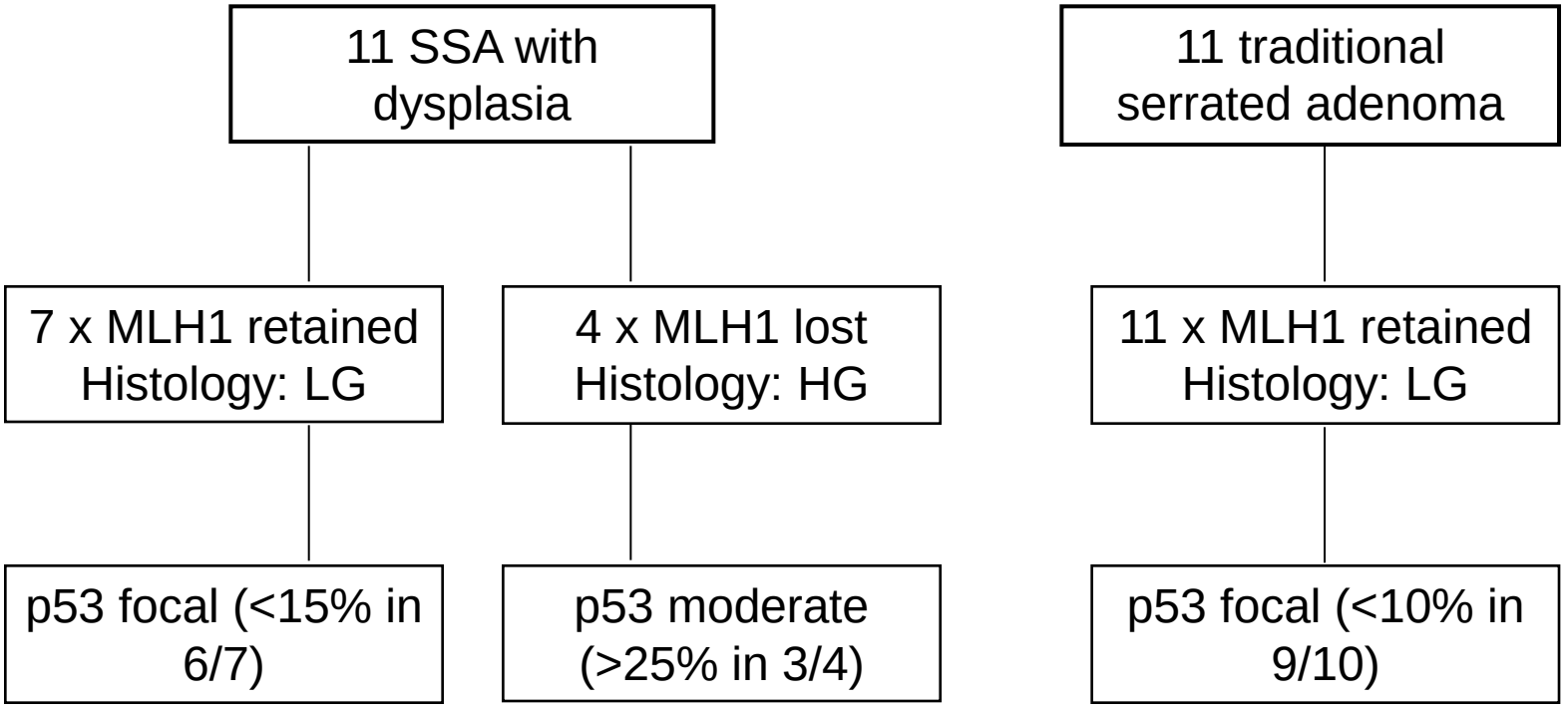
Medizinische Universität Graz



Parfitt and Driman Hum Pathol 2007

MLH1 and p53 Immuno-histochemistry in the DD SSA/P with Dysplasia (IEN) *vs* TSA

Medizinische Universität Graz



Overall, only 1/84 (1%) conventional adenomas but 3/25 (12%) SSA with dysplasia showed aberrant p53 expression ($p<0.04$).

p53 expression coincidenced with HG IEN

Parfitt and Driman Hum Pathol 2007

Beta-Catenin Immunohistochemistry

Medizinische Universität Graz

	Beta-Catenin membranous	Beta-Catenin nuclear			
HP (n=19)	19	0			
SSA/P (n=22)	13	9			

p<0.002

Wu et al. Am J Clin Pathol 2008

Beta-Catenin Immunohistochemistry

Medizinische Universität Graz

	Beta-Catenin membranous	Beta-Catenin nuclear	Keratin 7	Keratin 20	p53
HP (n=19)	19	0	16	19	0
SSA/P (n=22)	13	9	6	22	0

	Right	Left	
Positive (alle)	5	13	p<0.001
Negative (alle)	12	2	
Positive (SSA/P)	3	3	p<0.02
Negative (SSA/P)	16	0	

Wu et al. Am J Clin Pathol 2008

Beta-Catenin Immunohistochemistry

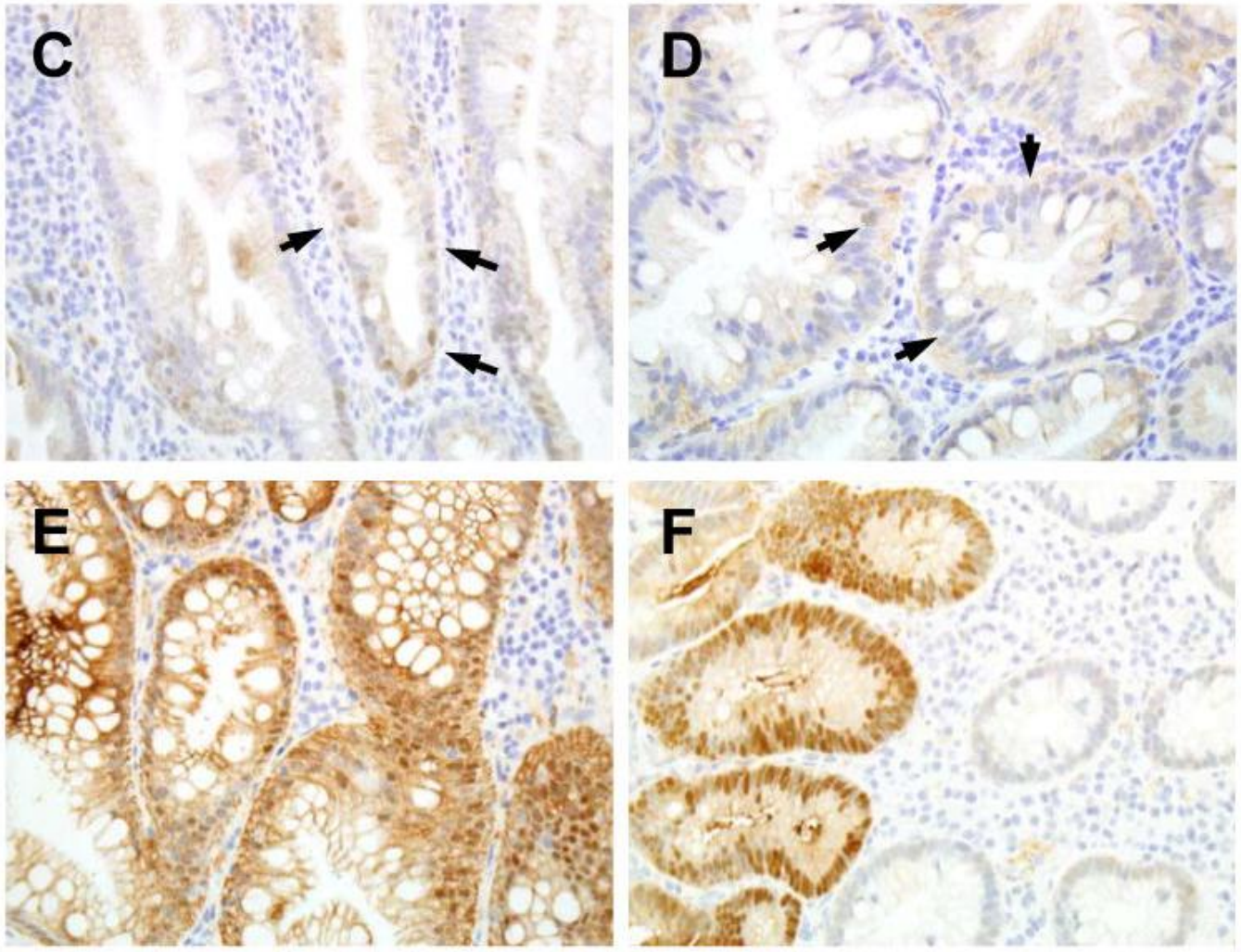
Medizinische Universität Graz

	n	Beta-Catenin (nuclear Expression)
HP	**12**	**0**
SSA/P	**54**	**35 (67%)**
TSA	1	4 (36%)
Tubular Adenoma	18	18 (100%)

	n	Beta-Catenin (nuclear Expression)
SSA/P without IEN	27	8 (29%)
SSA/P with LG IEN	27	27 (100%)

Yachida et al. Am J Surg Pathol 2009

Beta-Catenin Immunohistochemistry



Yachida et al. Am J Surg Pathol 2009

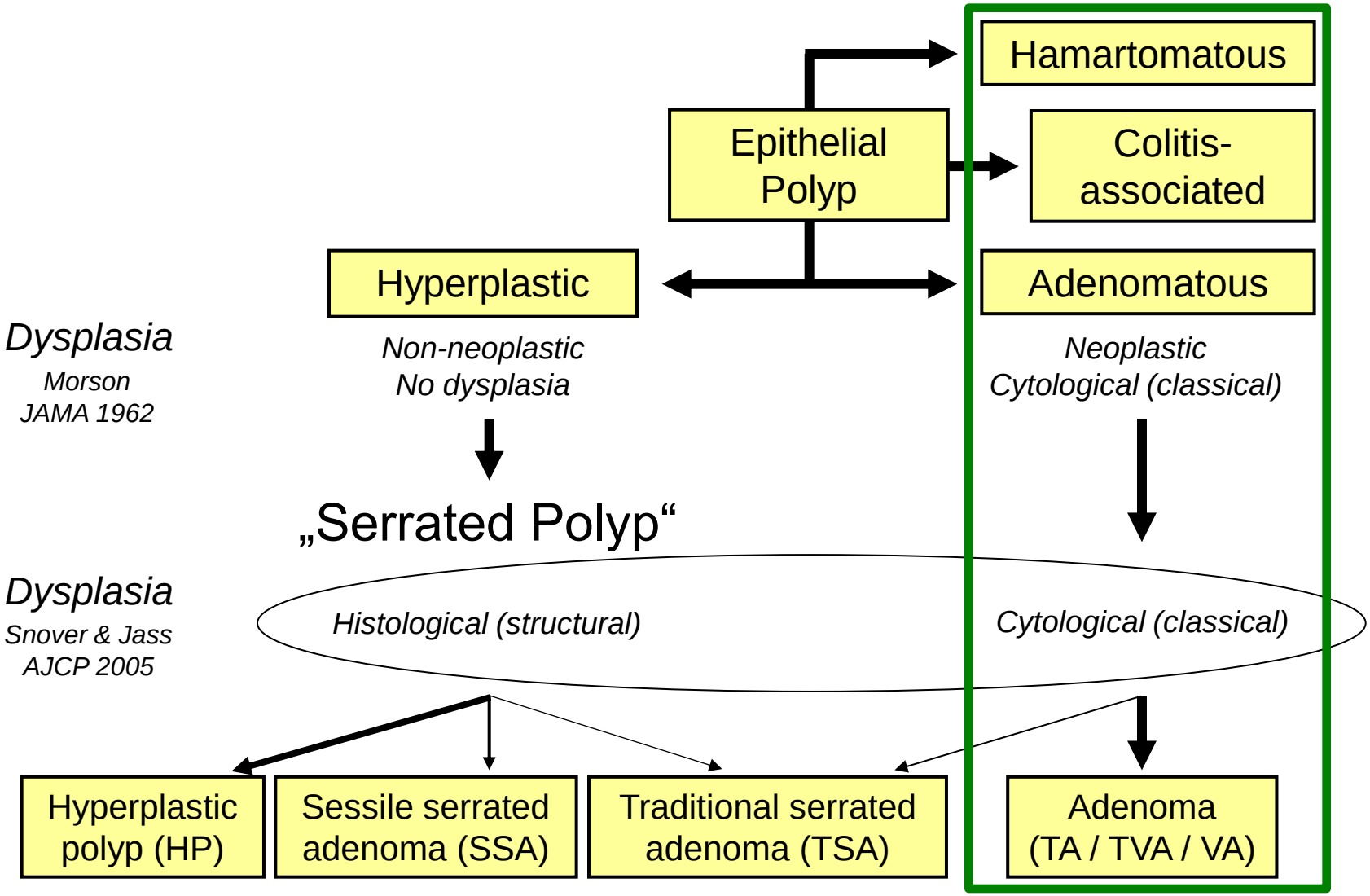
Take Home Message

Medizinische Universität Graz

- **Serrated lesions are new important players in the group of colorectal polyps**

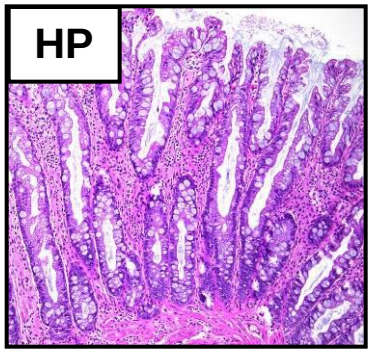
Classification of Colorectal Polyps

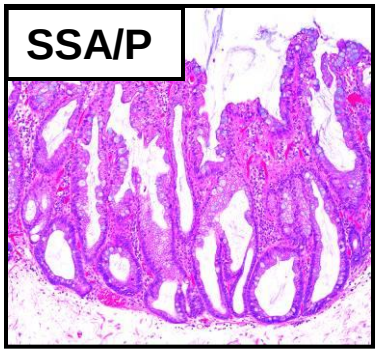
Medizinische Universität Graz

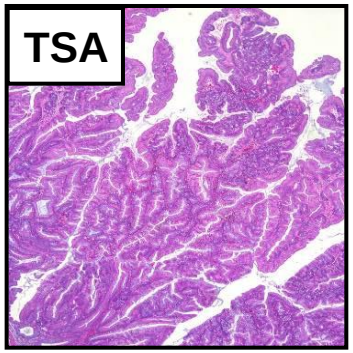


Differential Diagnosis

Medizinische Universität Graz







	HP	SSA/P	TSA
Frequency	80-90%	5-15%	1 (-5%)
Location	left > right	right > left	left > right
Size	<5 mm	>5-10 mm	>5 mm
Form	elevated	flat / sessile	elevated
Crypt Architecture			
Serration	upper third	basal	marked
Basal dilatation	-	+++	(+)
T&L crypt branching	-	+++	-
Inverted crypts	-	+++	-
Cytology			
Mitotic figures	lower third	middle third	upper third
Eosinophilia	(+)	++	+++
Dysplasia	-	-/(+)	+++

Take Home Message

Medizinische Universität Graz

- Serrated lesions are new players in the group of colorectal polyps
- **H&E staining remains to be the gold standard for differential diagnosis**
- **In selected cases, immunohistochemistry may help to establish the accurate diagnosis**
- **The serrated pathway involves a sequence of genetic and epigenetic alterations that lead to sporadic MSI-H CRC (CIMP phenotype)**

Join the European Network of Gastrointestinal Pathology (www.medunigraz.at/ENGIP)

Medizinische Universität Graz

Thank you very much for your kind attention!

Univ. Doz. Dr. med. Cord Langner
Institute of Pathology
Medical University of Graz / Austria
Chairman, Working Group of Digestive Disease Pathology
European Society of Pathology
cord.langner@medunigraz.at