ORIGINAL ARTICLE

Colonic Adenocarcinomas Harboring NTRK Fusion Genes

A Clinicopathologic and Molecular Genetic Study of 16 Cases and Review of the Literature

汇报人：贾旭春

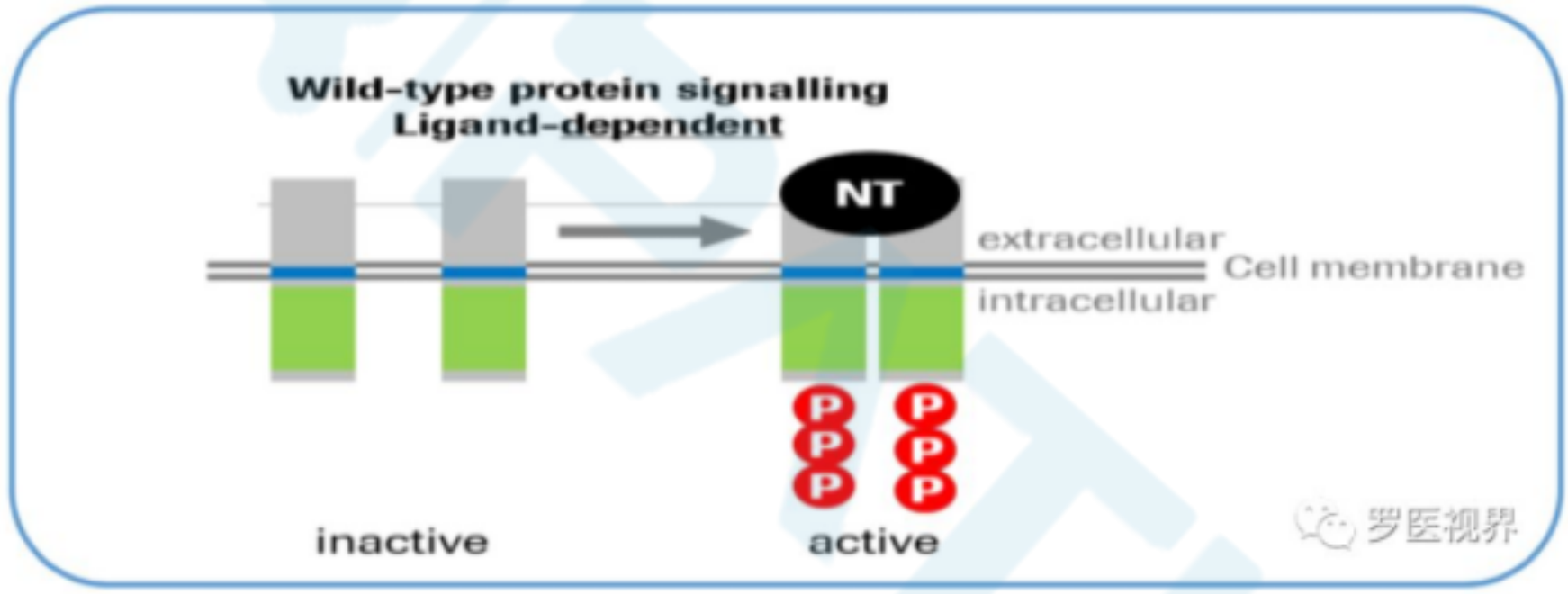
- 结肠癌（CRC）遗传异质性
- 致癌基因及抑癌基因突变导致的一系列基因组及表观基因组改变
- 致癌基因融合：最早肉瘤、淋巴瘤，癌
- 融合基因编码酪氨酸激酶受体—嵌合蛋白表达
- 诱导MAPK 及AKT下游信号通路 ---肿瘤发生

NTRK基因

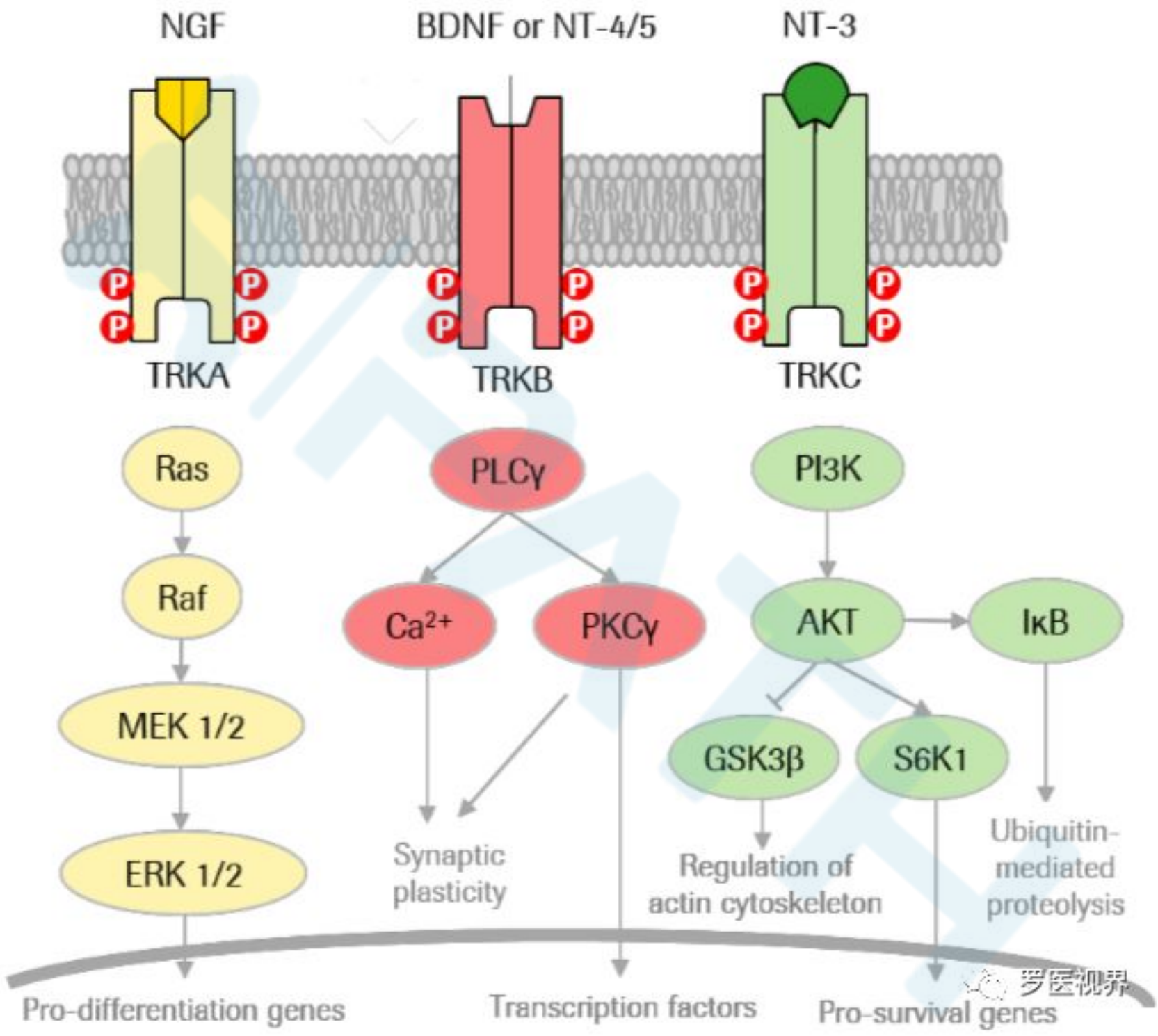
- Neurotrophic receptor tyrosine kinase
- 神经营养受体酪氨酸激酶

NRTK全称：神经营养型受体酪氨酸激酶（Neurotrophic Receptor Kinase）。NTRK基因包括三种亚型：NTRK1、 NTRK2、 NTRK3，分别位于不同染色体上。NTRK编码的蛋白叫做TRK蛋白，NTRK1、NTRK2、NTRK3分别编码TRKA、TRKB 、TRKC 三种蛋白[1-3]。

TRK蛋白



TRK蛋白是受体酪氨酸激酶的一种：包含膜外的配体结合区、跨膜区和细胞内激酶区域[1]。TRK蛋白与配体结合后，发生二聚体化，细胞内的酪氨酸激酶磷酸化后，向下游传递信号[4]。



正常人体环境中，不同TRK蛋白亚型与不同神经营养因子结合，调节中枢和外周神经系统中各类神经元的生长，分化和凋亡[5-8]。

NTRK基因融合

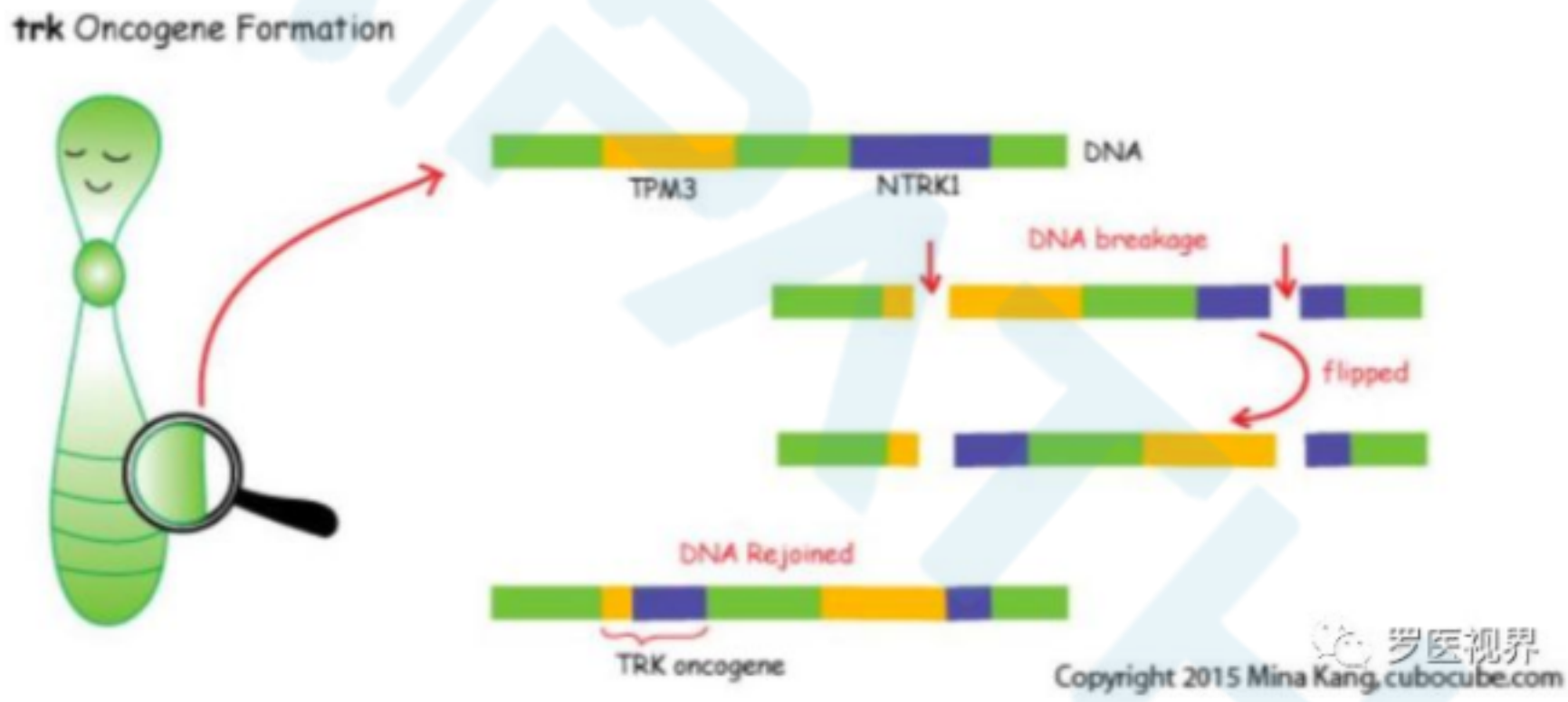

trk Oncogene Formation
DNA
TPM3
NTRK1
DNA breakage
flipped
DNA Rejoined
TRK oncogene
罗医视界
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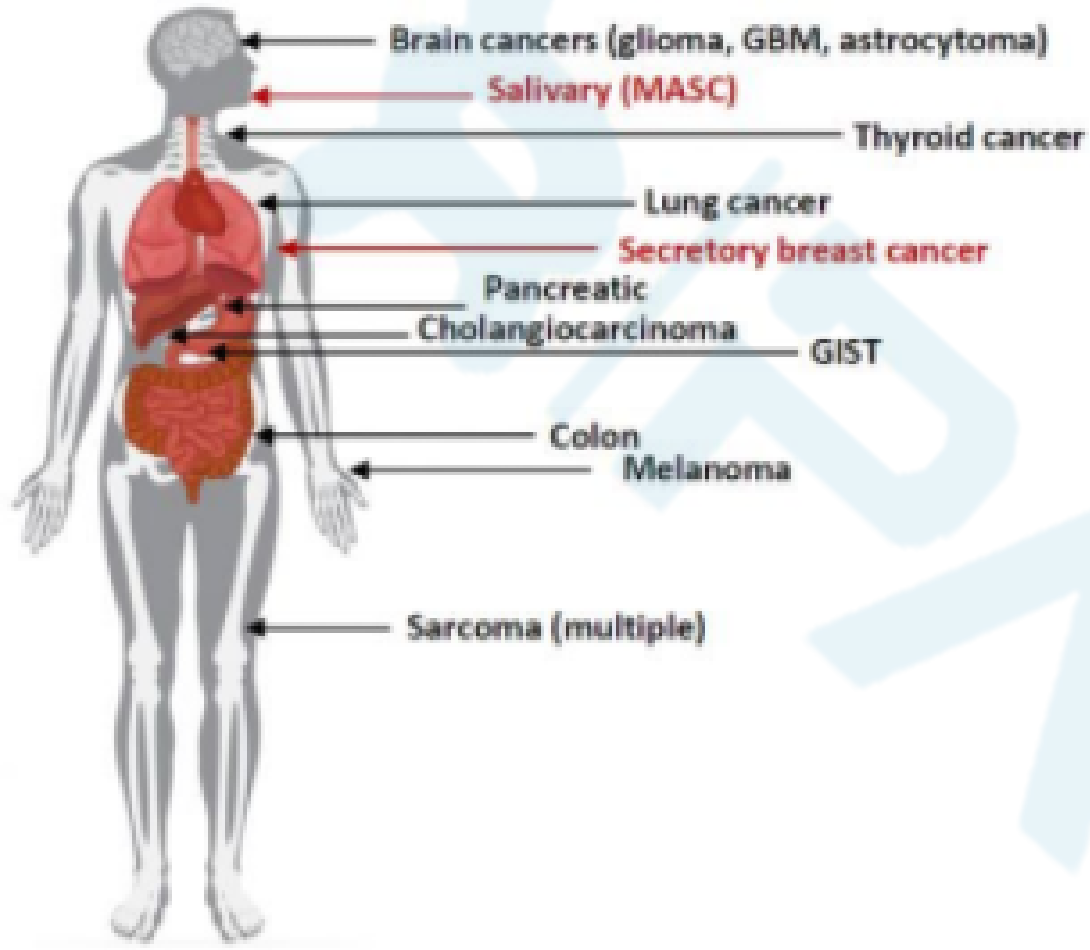
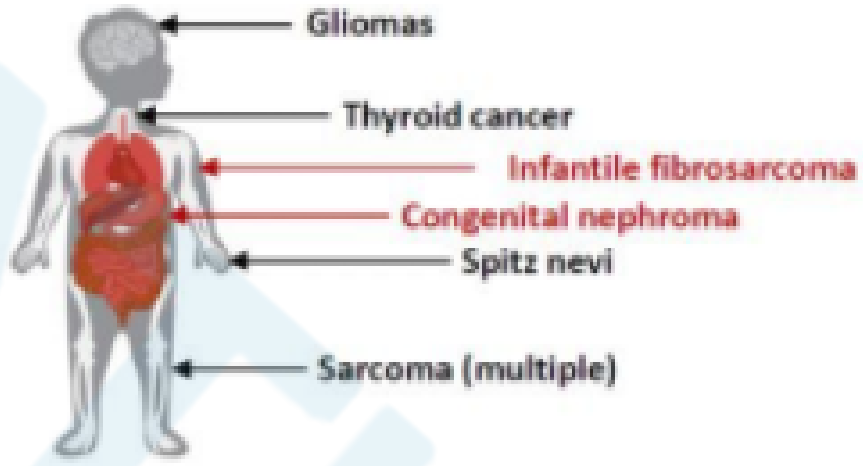
表 1　NTRK 基因融合与相关肿瘤 [11]

NTRK 基因	融合基因	融合位点	肿瘤类型
NTRK1 (1q21 ~ q22)	ARHGEF2	1q21 ~ q22	胶质母细胞瘤
	CD74*	5q32	非小细胞肺癌
	CHTOP	1q21.3	胶质母细胞瘤
	LMNA*	1q22	Spitzoid 肿瘤、结肠直肠癌、软组织肉瘤
	MPRIP*	17p11.2	非小细胞肺癌
	NFASC*	1q32.1	多形性成胶质细胞瘤
	SQSTM1*	5q35	非小细胞肺癌
	TFG*	3q12.2	乳头状甲状腺癌
	TP53	17p13.1	Spitzoid 肿瘤
	TPM3*	1q21.2	结肠直肠癌、乳头状甲状腺癌
	TPR*	1q25	乳头状甲状腺癌、结肠直肠癌
NTRK2 (9q22.1)	AFAP1	4p16	低级别神经胶质瘤
	NACC2	9q34.3	毛细胞性星形细胞瘤
	PAN3	13q12.2	头颈鳞状细胞癌
	QKI	6q26	毛细胞性星形细胞瘤
	TRIM24	7q32 ~ q34	非小细胞肺癌
NTRK3 (15q25)	ETV6	12p13	先天性纤维肉瘤、先天性中胚层肾瘤、分泌型乳腺癌、急性髓细胞样白血病、类似乳腺分泌性癌





Common cancer with low TRK fusion frequency

Rare cancer with high TRK fusion frequency

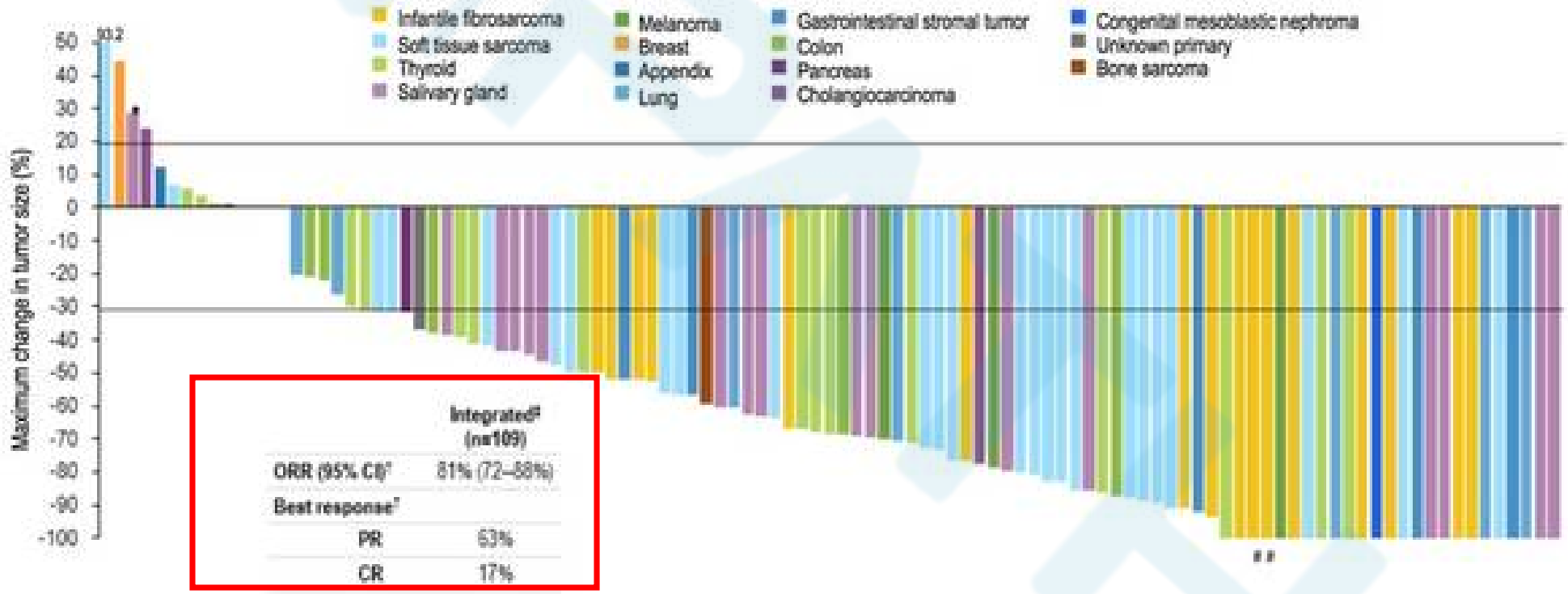
Estimated 1,500–5,000 patients harbor TRK fusion-positive cancers in the United States annually

临床意义

- TRK抑制剂—拉罗替尼
- 2018年5月 FDA获批
- 治疗含有NTRK融合的进展期及转移性肿瘤

效果明显

Integrated dataset: Larotrectinib is efficacious regardless of tumor type



	Integrated[F] (n=109)
ORR (95% CI)[*]	81% (72–88%)
Best response[*]	
PR	63%
CR	17%

[F]Includes 9 unconfirmed PRs pending confirmation; does not include 13 patients continuing on study and awaiting initial response assessment
[*]Patient had TRKC solvent front resistance mutation (G623R) at baseline due to prior therapy; #Surgical CR; [†]RECIST 1.1
Note: Two patients not shown here. These patients discontinued treatment prior to any post-baseline tumor measurements.
CR, complete response; ORR, objective response rate; PR, partial response

MUNICH 2018 ESMO congress

Investigator response assessments, as of 30 July 2018

- CRC最早35年前TPM3- NTRK1报道
- 少量NTRK融合报道，但临床病理数据有限

材料与方法

- 评估7008例来自欧洲、日本及美国结肠癌
- 组织芯片
- IHC： pan-Trk
  MMR（ MLH1, PMS2, MSH2, MSH6 ）
  CDX2, CK7, CK20, Ki-67, MUC2, p53 , β-catenin
  PD-L1
- NGS（RNA）
- MLH1启动子甲基化

结果

- 7008例中16例pan-Trk 阳性（0.23%）
- 15例进行NGS

✓TPM3-NTRK1　9例

✓ LMNA-NTRK1　3例

✓ TPR-NTRK1　　2例

✓ EML4 -NTRK3　1例

免疫组化

TABLE 1. Type of NTRK Fusion and pan-Trk Expression Pattern Identified in 15 Colon Carcinomas

Fusion Gene	No. Tumors	Membrane pan-Trk Staining	Cytoplasmic pan-Trk Staining	Nuclear pan-Trk Staining
TPM3-NTRK1	9	+++	+++	–
LMNA-NTRK1	3	+ or +/–	+++	Focal or scattered cells
TPR-NTRK1	2	+	++	–
EML4-NTRK3	1	–	+	–

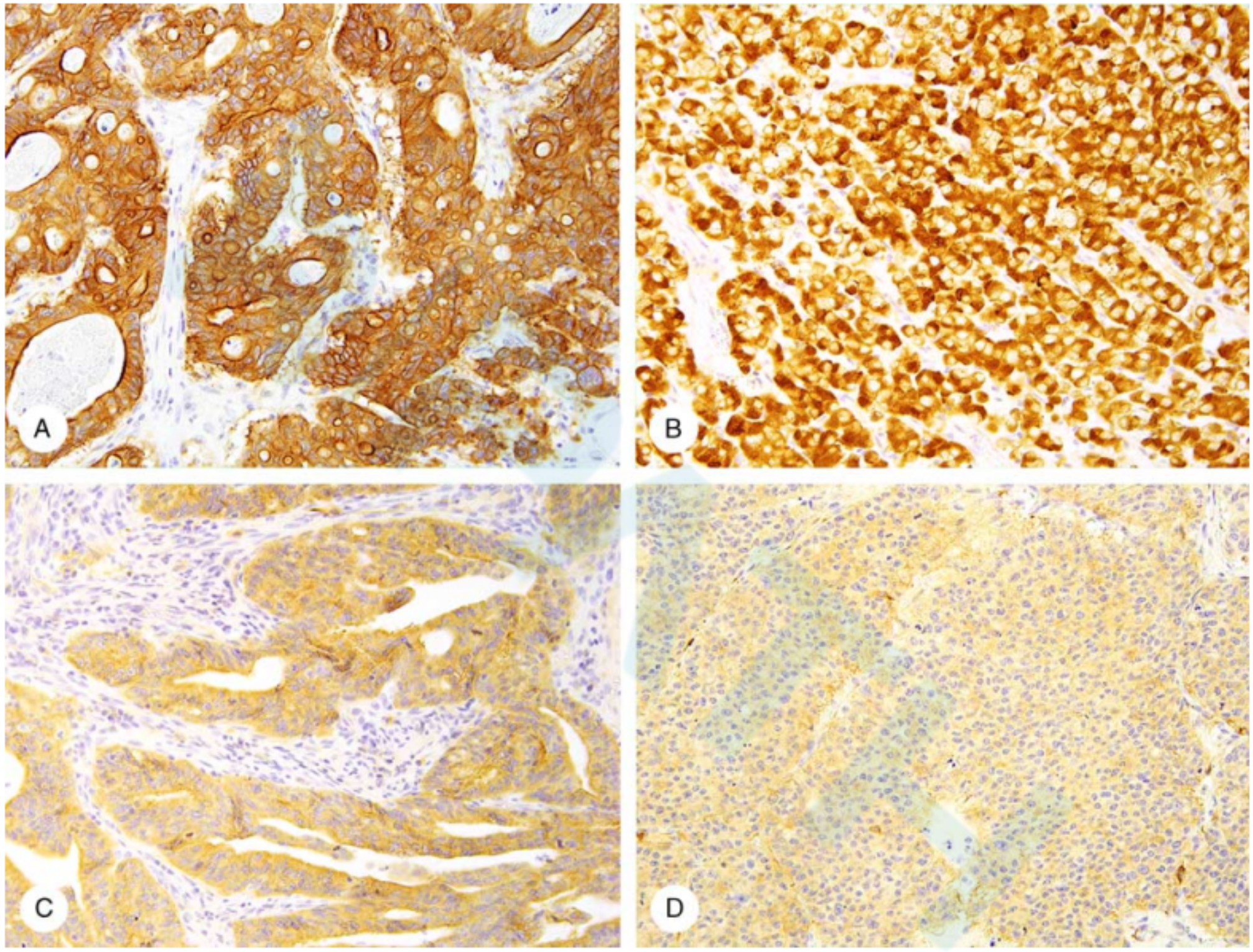


FIGURE 1. Trk-positive immunohistochemistry in colon cancers. TPM3(e8)-NTRK1(e10) fusion tumor (case 8) displayed strong, diffuse cytoplasmic and membrane staining (A); LMNA(e10)-NTRK1(e11) fusion tumor (case 14) with an area of strong nuclear staining and weaker cytoplasmic staining (B); TPR(e21)-NTRK1(e10) fusion tumor (case 5) showed weaker cytoplasmic and membrane staining than TPM3-NTRK1 and LMNA-NTRK1 fusion tumors and lacked nuclear immunoreactivity (C); EML4(e2)-NTRK3(e14) fusion tumor (case 12) displayed weak but distinct cytoplasmic staining and no membrane or nuclear immunoreactivity (D).

临床病理特征

TABLE 2. Clinical Characteristics of 16 Colon Cancers With Positive pan-Trk Immunohistochemistry

Case	Age (y)	Sex	Tumor Site in Colon	Staging System (pTNM, Dukes C)	Follow-Up	NTRK Fusion Gene
1	54	Female	Cecum	pT4aN2bM1b	DOD (1 mo)*	TPM3(e8)-NTRK1(e10)
2	68	Female	Cecum	pT3N0M0	ANED (1 y 5 mo)†	LMNA(e4)-NTRK1(e10)
3	46	Female	Ascending	Dukes C‡	ANED (17 y)§	Unknown‖
4	50	Female	Hepatic flexure	pT3N0M0	ANED (1 y 10 mo)§	TPM3(e8)-NTRK1(e13)
5	53	Female	Hepatic flexure	pT3N1aM0	ANED (2 y 4 mo)§	TPR(e21)-NTRK1(e10)
6	63	Male	Hepatic flexure	pT3N1M0	ANED (7 y)‡	TPM3(e8)-NTRK1(e13)
7	86	Female	Hepatic flexure	pT3N0M0	DOC (7 d)†	LMNA(e11)-NTRK1(e8)
8	77	Female	Transverse colon	pT3N0M0	NA	TPM3(e8)-NTRK1(e10)
9	84	Male	Transverse colon	pT3N0M0	DOC (2 y)†	TPM3(e8)-NTRK1(e10)
10	63	Female	Splenic flexure	pT3N0M0	ANED (3 y 9 mo)§	TPM3(e8)-NTRK1(e10)
11	68	Female	Splenic flexure	pT3N0M0	ANED (11 mo)‡	TPR(e21)-NTRK1(e10)
12	71	Female	Splenic flexure	pT3N0M0	ANED (12 y 4 mo)‡	EML4(e2)-NTRK3(e14)
13	56	Female	Descending	pT4aN2bM0	NA	TPM3(e8)-NTRK1(e10)
14	62	Female	Descending	pT4aN2bM0	ANED (1 y 1 mo)§	LMNA(e10)-NTRK1(e11)
15	71	Male	Descending	pT3N0M0	NA	TPM3(e8)-NTRK1(e10)
16	70	Female	Rectosigmoid junction	pT4aN0M0	NA	TPM3(e8)-NTRK1(e10)

*Adjuvant chemotherapy status unknown.
†No adjuvant chemotherapy.
‡6 cm tumor, metastases in local lymph nodes.
§Adjuvant chemotherapy.
‖RNA failed quality control test.
ANED indicates alive, no evidence of disease; DOC, died of unknown causes; DOD, died of disease; NA, not available.

临床病理特征

- 大部为女性（13/46，81%）
- 女性中位年龄63岁，男性71岁
- 12例T3，4例T4分期
- 5例淋巴结转移，1例远处转移（肝、肺）
- 12例随访

✓1例有远处转移 1月死亡

✓2例年龄大（ 84、86 ），7天、24月死亡

✓9例无病生存11月-17年

组织学特征

TABLE 3. Histopathologic Characteristics of 16 Colon Cancers With Positive pan-Trk Immunohistochemistry

Case	Degree of Glandular Differentiation	Solid Growth Area	Mucinous Component	TILs
1	Moderate to poor	Focal	No	Low*
2	Poor	Extensive	Focal	High†
3	Moderate to poor	No	Focal	Low
4	Moderate to poor	Focal	Extensive‡	Low
5	Moderate to poor	No	Focal	High
6	Moderate	No	No	Low
7	Poor (medullary subtype)	Extensive	No	Low
8	Moderate to poor	Focal	Focal	High
9	Moderate to poor	No	No	High
10	Moderate to poor	No	No	High
11	Moderate to poor	Focal§	Extensive‡	Low
12	None	All	No	High
13	Moderate to poor	Focal	Focal	High
14	Poor	Focal‖	Extensive‡	Low
15	Moderate to poor	No	Yes	High
16	Poor	Extensive	Focal	High

*<2 TIL/HPF.
†≥2 TIL/HPF.
‡Luminal.
§Minimal.
‖Sheets of signet ring cells.
HPF indicates high-power fields.

- 大多中-低分化腺癌（中11例，低4例）
- 8例局灶-大量实性区域，1实性带状生长模式
- 8例可见黏液成分，1例片状印戒细胞
- 9例高水平TILS
- 所有均有脉管侵犯

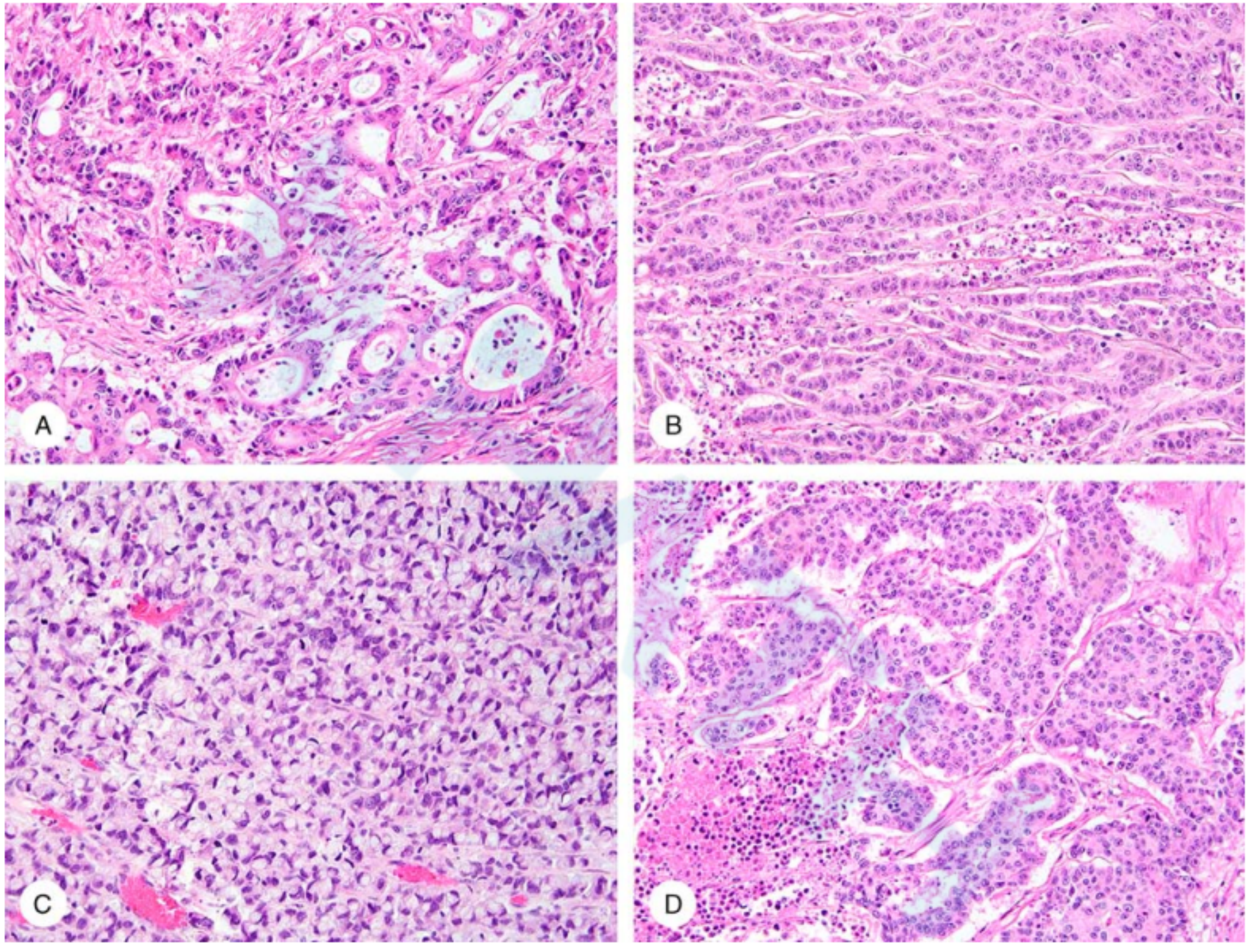


FIGURE 2. Histologic features of Trk-positive colon cancers. TPM3(e8)-NTRK1(e10) fusion tumor (case 13) with moderate to poor differentiation (A); LMNA(e11)-NTRK1(e8) fusion tumor (case 7) with solid areas displaying ribbon-like growth pattern (B); LMNA (e10)-NTRK1(e11) fusion tumor (case 14) with prominent sheets of signet ring cells (C); EML4(e2)-NTRK3(e14) fusion tumor (case 12) showed a vague nested growth pattern (D).

免疫组化结果

TABLE 4. Immunophenotype of 16 Colon Cancers With Positive Trk Immunohistochemistry

Case	MLH1	PMS2	MSH2	MSH6	CK7	CK20	CDX2	MUC2
1	Retained	Retained	Retained	Retained	Diffuse	Diffuse	Diffuse	No exp
2	Loss	Loss	Retained	Retained	Diffuse	No exp	No exp	Focal
3	Loss	Loss	Retained	Retained	No exp	No exp	Diffuse	No exp
4	Loss	Loss	Retained	Retained	No exp	Focal	Diffuse	Focal
5	Loss	Loss	Retained	Retained	Diffuse	Focal	Diffuse	No exp
6	Retained	Retained	Retained	Retained	No exp	Diffuse	Diffuse	No exp
7	Loss	Loss	Retained	Retained	No exp	No exp	Focal	No exp
8	Loss	Loss	Retained	Retained	No exp	Focal	Diffuse	No exp
9	Loss	Loss	Retained	Retained	No exp	Focal	Diffuse	Focal
10	Loss	Loss	Retained	Retained	No exp	No exp	No exp	Sc
11	Loss	Loss	Retained	Retained	No exp	Focal	Diffuse	Sc
12	Loss	Loss	Retained	Retained	Diffuse	Sc	Diffuse	Sc
13	Loss	Loss	Retained	Loss	No exp	Sc	Diffuse	No exp
14	Retained	Retained	Retained	Retained	Focal	Diffuse	Diffuse	Diffuse
15	Loss	Loss	Retained	Retained	No exp	No exp	Diffuse	Diffuse
16	Loss	Loss	Retained	Retained	No exp	No exp	Focal	No exp

No exp indicates No expression; Sc, scattered cells.

免疫组化结果

- 13/16（ 81% ）MLH1及PMS2缺失
- 1例MLH6缺失（伴 MLH1及PMS2缺失）
- MLH2均无缺失
- CK20（9/16）、CK7（5/16）表达
- CDX2大部阳性，4例局灶、全部缺失
- MUC2 8例缺失

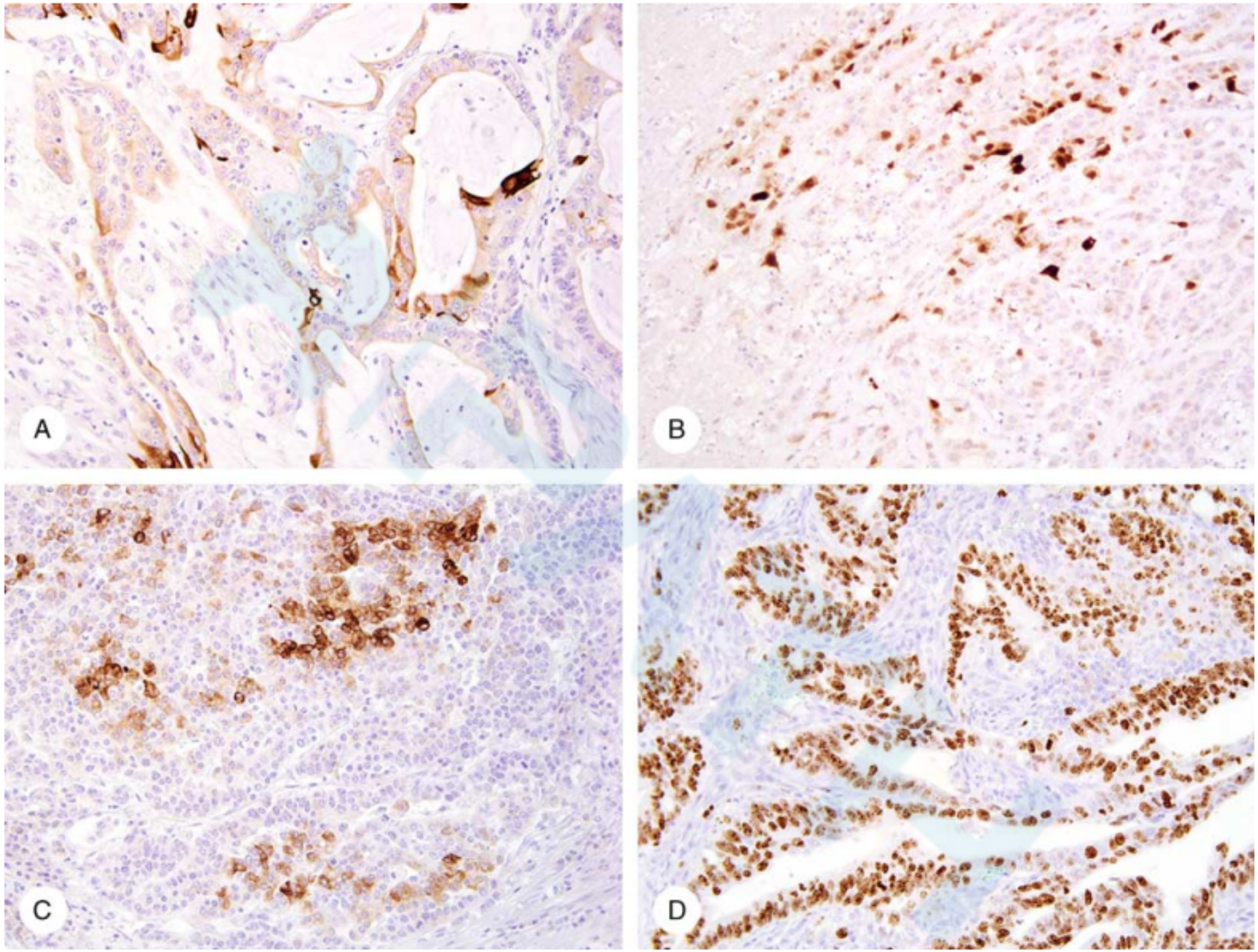


FIGURE 3. Immunohistochemistry of Trk-positive colon cancers. Focal CK20 expression (A) in TPM3(e8)-NTRK1(e10) fusion tumor (case 15); focal CDX2 expression (B) in LMNA(e11)-NTRK1(e8) fusion tumor (case 7); focal MUC2 expression (C) in LMNA(e4)-NTRK1(e10) fusion tumor (case 2); diffuse (near 100%) Ki-67 expression (D) in TPR(e21)-NTRK1(e10) fusion tumor (case 5).

免疫组化

- 4例P53核弥漫表达，1例完全缺失，余不同程度核表达
- 所有病例β-catenin膜及浆表达，其中1例有明显核堆积（MMR缺失）
- PD-L1肿瘤浸润免疫细胞多少不等表达，无肿瘤细胞或极少表达

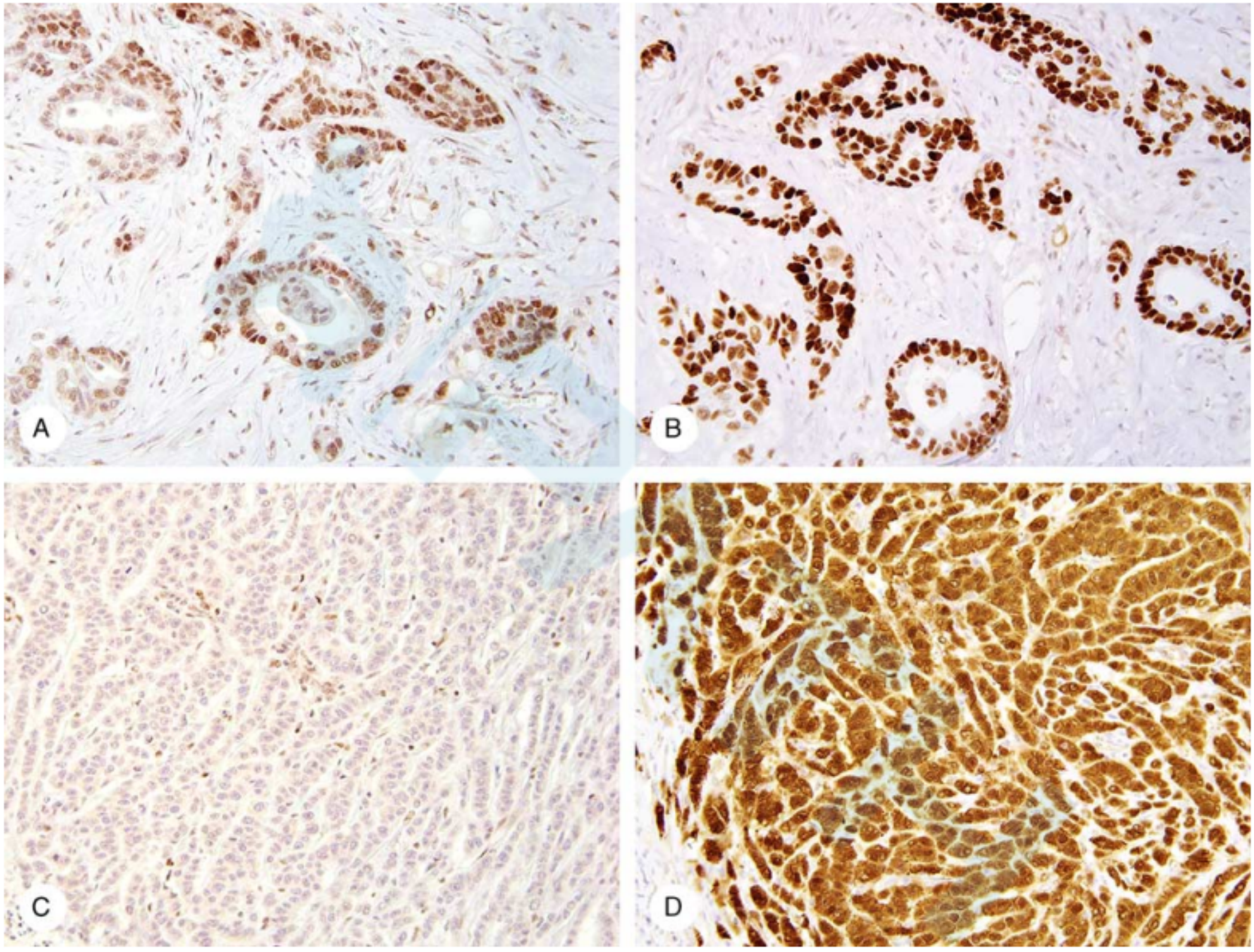


FIGURE 4. Involvement of p53 and β-catenin pathways in Trk-positive colon cancers. TPM3(e8)-NTRK1(e10) fusion tumor (case 1) with retained MLH1 expression (A) harbored a *TP53* mutation (p.Met246Arg) and displayed diffuse p53 immunostaining (B); LMNA(e11)-NTRK1(e8) fusion tumor (case *7*) with loss of MLH1 expression (C) harbored an *APC* mutation (p.Cys1502Ter) and displayed nuclear β-catenin accumulation (D).

甲基化

2例显示MLH1启动子甲基化

突变

- 9例-409个基因
- 1例—50个基因

TGFβ通路

Wnt/β-catenin通路

PI3K-AKT/MTOR通路

TABLE 5. Mutated Oncogenes and Tumor Suppressor Genes in *NTRK* Fusion Colon Cancers Reported in This Study

Gene ID	Mutation	Predicted Functional Consequences*	Case
ACVR2A	p.Asp386fs	NA	2
AKT1	p.Ser240Pro	Pathogenic	10
AMER1	p.Arg531Ter	Neutral	8
AMER1	p.Gly140Asp	NA	16
AMER1	p.Val305fs	NA	2
APC	p.Cys1502Ter	Pathogenic	7
APC	p.Pro750Ser	Pathogenic	16
APC	p.Cys1289Tyr	Pathogenic	11
CTNNB1	p.Arg225Cys	Pathogenic	8
CTNNB1	p.Lys354Asn	Pathogenic	2
FBXW7	p.Arg479Gln	Pathogenic	13
FBXW7	p.Trp446Ter	Pathogenic	11
MTOR	p.Ala1792Val	Pathogenic	2
PIK3C2B	p.Arg727Trp	Pathogenic	15
PIK3CD	p.Val370fs	NA	8
PIK3CD	p.Gly245Ser	Pathogenic	15
PIK3R2	p.Val54Met	Pathogenic	5
PTEN	p.His272fs	Pathogenic	1
PTEN	p.Asn323fs	NA	10
TGFBR2	p.Trp529Ter	NA	16
TP53	p.Met246Arg	Pathogenic	1
TP53	p.Pro72Arg	Neutral	11
TP53	p.Cys176Tyr	Pathogenic	15

*On the basis of the FATHMM, SIFT, PolyPhen scores predicting functional consequences of coding variants.

NA indicates not available.

突变

- 均无MLH1, PMS2, MSH2，MSH6 突变
- 均无BRAF , K-RAS, N-RAS，PIK3CA突变（典型CRC驱动基因）

- 5例 AKT1, MTOR , PTEN, PIK3CD, PIK3R2, PIK3C2B ----- PI3K-AKT/MTOR通路
- 5例APC , AMER1，CTNNB1 -----Wnt/β-catenin通路
- 2例ACVR2A 、TGFBR2-----TGFβ通路

- 3例TP53
- 2例FBXW7—抑癌基因

讨论

pan-Trk

- 7008例中16例阳性表达0.23%， 15例NTRK基因融合
- 较高特异性
- 以前报道类似
- 敏感性无法评估（阴性病例无基因型）
- 有研究1272例CRC二代测序NTRK融合0.2%

讨论

- NTRK1融合基因： LMNA, PLEKHA6, SCYL3, TPM3, TPR
- TPM3-NTRK1 最普遍（60%）
- 甲状腺乳头状癌、 spitz 样黑色素肿瘤、肝内胆管癌，胶质母细胞瘤、儿童高级别胶质瘤、肺非小细胞癌，软组织及子宫肉瘤等
- LMNA-NTRK1（ 20% ） 及 TPR-NTRK1（13%）

TABLE 6. Types of NTRK1 Fusions Described in 33 CRCs in This (n = 15) and Previously Published Studies*[8–16,21,26]

	LMNA					PLEKHA6	SCYL3	TPM3		TPR	
NTRK1	**e4**	**e6**	**e9**	**e10**	**e11**	**e22**	**e11**	**e7**	**e8**	**e16**	**e21**
e8								2§			
e9									1	1	
e10	1					1		6	10		2
e11	1			2	1†				1‡		
e12		1					1				
e13									2		
Subtotal		6				1	1	22		3	
Total						33					

*Excluding 16 cases harboring 11 TPM3-NTRK1, 3 LMNA-NTRK1, and 2 TPR-NTRK1 fusions reported without specific data on fusion breaks.[15,16]
†Two fusion transcripts, LMNA(e10)-NTRK1(e11) and LMNA(e11)-NTRK1(e11) formed due to alternative splicing.
‡Two fusion transcripts, TPM3(e8)-NTRK1(e11) and TPM3(e8)-NTRK1(e12) in 1 tumor.
§Intraexonic break in 1 case.

讨论

- NTRK3融合在CRC中罕见
- 仅有COX5A, EML4, ETV6，VPS18
- ETV6-NTRK3最常见
- 最先在婴儿纤维肉瘤及先天性中胚层肾瘤命名
- NTRK3 融合：分泌型乳腺癌、唾液腺癌、甲状腺乳头状癌、急性髓系白血病、黑色素肿瘤、胶质瘤等

TABLE 7. Types of NTRK3 Fusions Described in 10 CRCs in This (n = 1) and Previously Published Studies*[4,15,16,26] and Available at The Cancer Genome Atlas

	COX5A	ELM4	ETV6	VPS18
NTRK3	e1	e2	e5	e11
e14		2		
e15	1		6	
e18				1
Total		10		

*Excluding 4 cases harboring ETV6-NTRK3 fusions reported without specific data on fusion breaks.[15,16]

大部NTRK融合CRC显示微卫星不稳定特征

- 女性优势、右半结肠、黏液分化、高TILS
- 本文13/16MMR缺失
- 均无MLH1, PMS2, MSH2，MSH6 突变
- 2例 MLH1启动子甲基化
- 散发病例

- 无BRAF 突变（典型CRC伴MMR缺失）
- K-RAS, N-RAS及 PIK3CA均无突变
- 缺乏BRAF，N-RAS突变有类似报道

- Wnt/β-catenin 及 p53通路的基因 (7/10)例基因突变共存
- β-catenin 核聚集P53核表达---功能性修饰
- 文献显示APC及TP53突变在NTRK融合CRC中
- TGFβ 通路成份2例MMR检出-常见事件
- PI3K-AKT/MTOR通路而不是PIK3CA
- 2例FBXW7 （P53相关的抑癌基因）
- FBXW7失活仅次于TP53突变

肿瘤部位

- 有研究Trk阳性CRC倾向左半结肠
- 最近研究显示肿瘤部位更能突出分子组成不同
- 研究NTRK1融合及NTRK3融合分布不同

✓NTRK1融合更倾向左半

✓NTRK3融合左右相同

PD-L1

- PD-L1肿瘤细胞无强而弥漫表达，局灶阳性
- 相关研究不明
- 有报道TPM3-NTRK1肿瘤伴PD-L1扩增及弥漫强表达
- 抗PD-L1治疗有效病例，局部PD-L1阳性

IHC异常表达

- 缺失CK7, CK20，MUC2表达
- 偶尔CDX2缺失
- 模式提示高侵袭性、低分化，易早期复发
- 但生存期较长（45月-17年）
- 报道NTRK融合CRC显示4-5年随访，未化疗
- 有预后差报道

NTRK融合CRC

- 类似MMR缺失特征
- 区分：靶向治疗抑制致癌TRK融合蛋白

2步骤

- TRK免疫组化
- 阳性病例进行分子基因检测
- 晚期和/或转移性病例且伴有BRAF/RAS 野生型

2020 NCCN指南更新

- 建议KRAS、NRAS、 BRAF野生型伴有MMR缺陷/MSI的患者进行NTRK融合基因检测
- 可用免疫组化筛选、快速经济
- 阳性进一步分子验证

谢 谢!