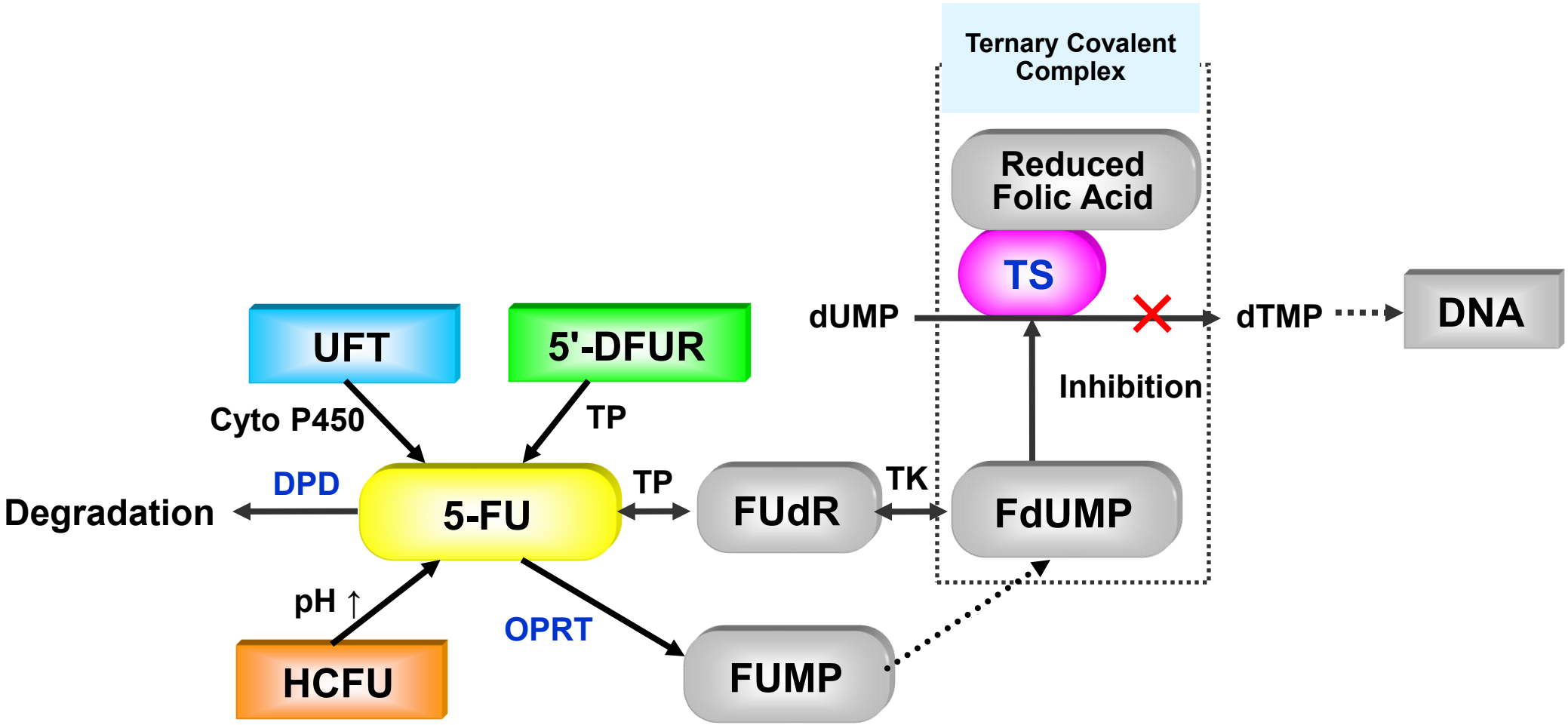
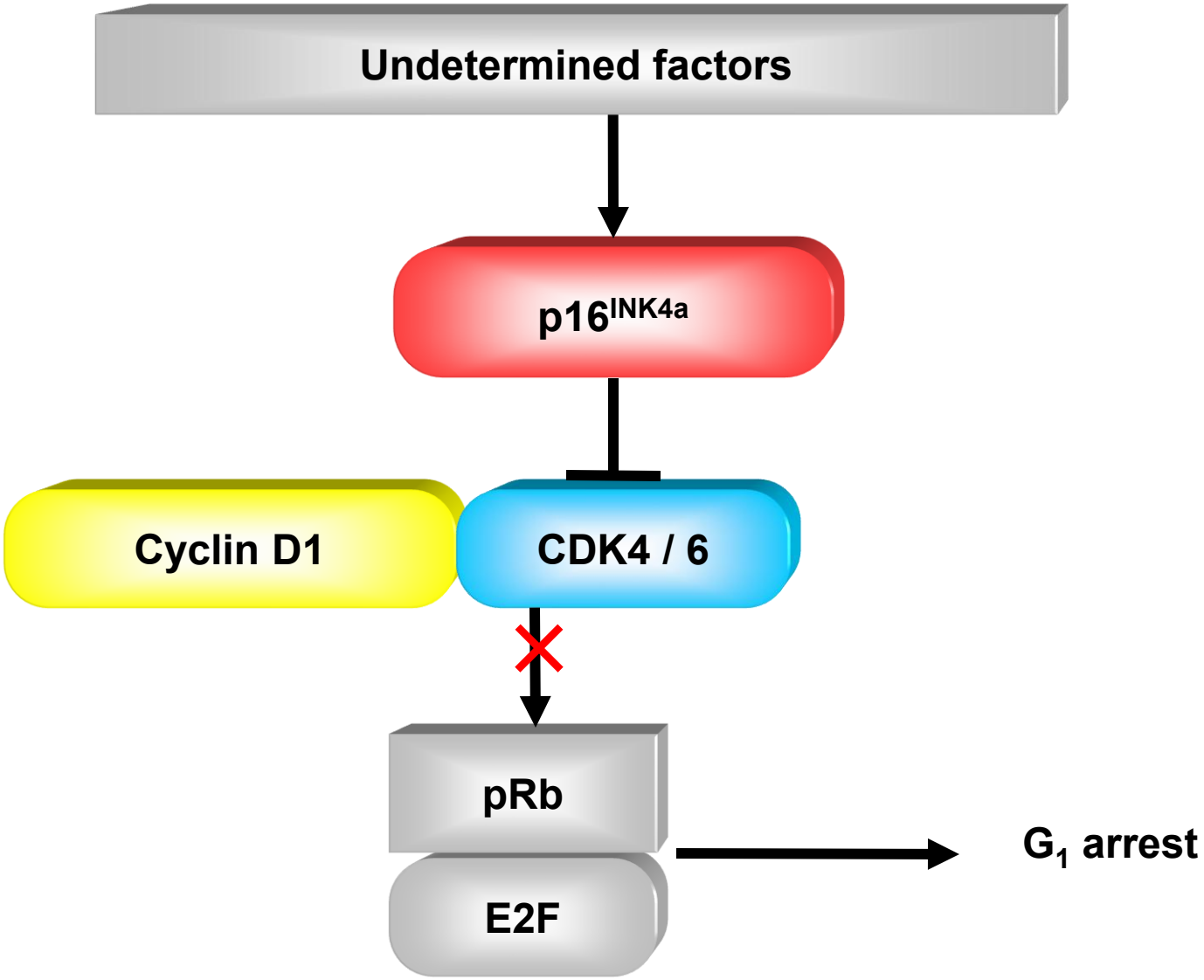
Anticipation of the resistance and susceptibility to 5-FU chemotherapy: Choice of taylor-made medicine for colorectal cancer

A high level of immunohistochemical expression of thymidylate synthase (TS) can be a predictor of 5-fluorouracil (5-FU) resistance, and the combination of a low level of TS expression and induction of $p16^{INK4a}$ after chemotherapy implicate chemosensitivity. Namely, a 5-FU-based regimen can be chosen as a first-line chemotherapy for low TS expressors, while TS-high cancer should be treated with anti-cancer agents acting through different mechanisms.

Ref.: Kamoshida S, et al. Immunohistochemical evaluation of thymidylate synthase (TS) and $p16^{INK4a}$ in advanced colorectal cancer: implication of TS expression in 5-FU-based adjuvant chemotherapy. Jpn J Clin Oncol 2004; 34(10): 594-601. doi: 10.1093/jjco/hyh113



DNA disturbance pathways by 5-fluorouracil (5-FU) and 5-FU prodrugs



Cell cycle regulation via $p16^{INK4a}$／Rb

Cases: advanced colorectal cancers treated with preoperative chemotherapy using 5-FU prodrugs (UFT or 5‘-DFUR) for 2 weeks: 37 cases

Untreated control cases: 31 cases

Methods: Formalin-fixed, paraffin-embedded biopsy and surgical specimens of colorectal adenocarcinoma (using the amino acid polymer method with the heat-assisted epitope retrieval sequence).

Target antigens:

1) thymidylate synthase (TS)
2) $p16^{INK4}$
3) cyclin-dependent kinase 4 (CDK4)
4) cyclin D1
5) dihydropyrimidine dehydrogenase (DPD)
6) orotate phosphoribosyltransferase (OPRT)

Histological effects of preoperative chemotherapy with 5-FU

Grade 0: No effect

Grade 1: Mild effect

1a: Necrosis seen in less than one third of cancer

1b: Necrosis seen in one third to two thirds of cancer

Grade 2: Significant effect (Necrosis seen in more than two thirds of cancer

Grade 3: Marked effect (Viable cancer cells lost)

Judgment of treatment responsiveness:

Responders: Grade 1b and Grade 2 (no lesions with Grade 3 effect)

Non-responders: Grade 0 and Grade 1a

Judgment criteria of immunostaining for TS, p16^{INK4a}, CDK4 and cyclin D1

Low expressors:

TS and p16^{INK4a}: Negative or Less than 30% of cancer cells expressed

CDK4 and cyclin D1: negative or Less than 10% of cancer cells expressed

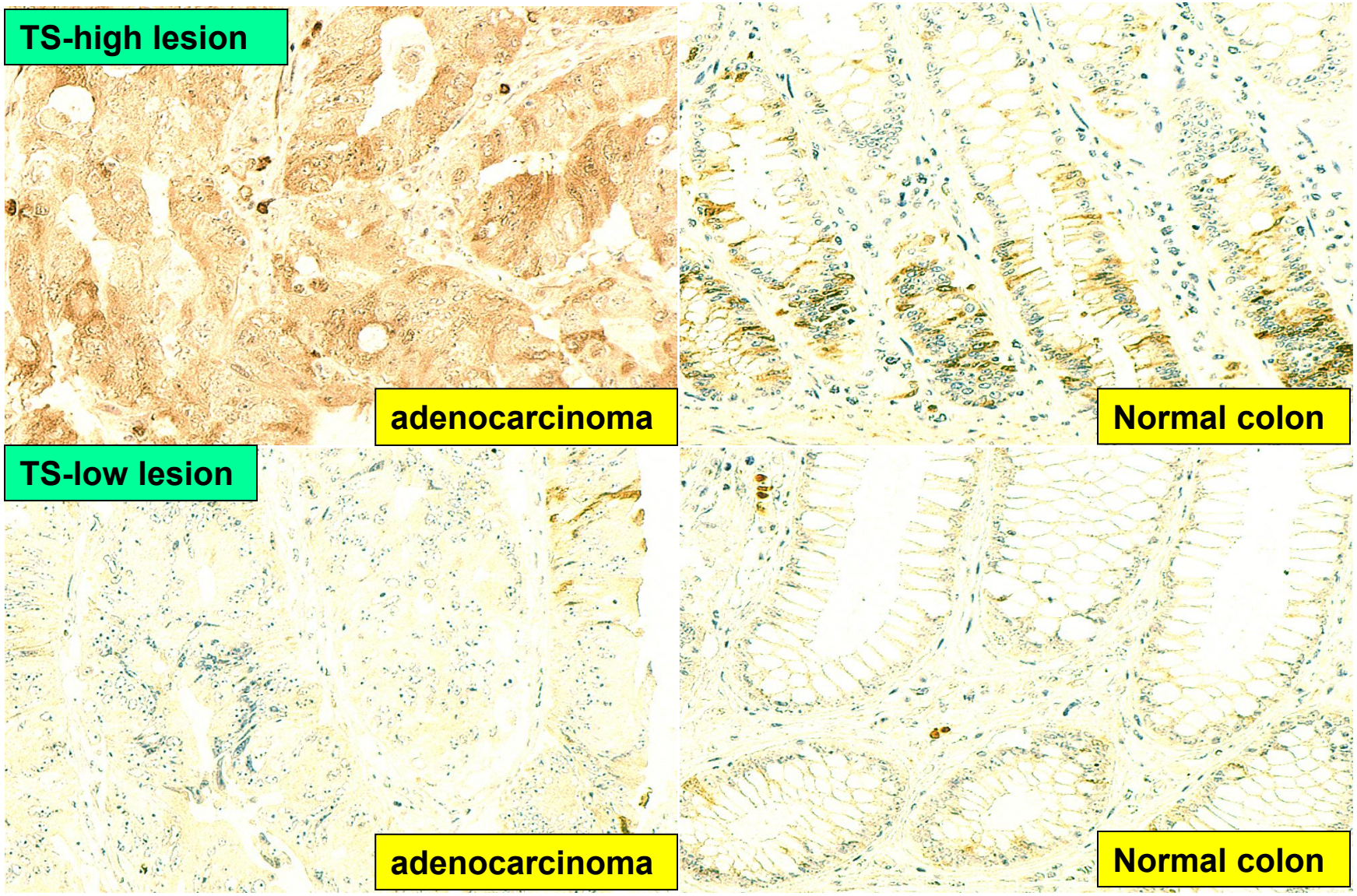
High expressors:

TS and p16^{INK4a}: More than 30% of cancer cells expressed

CDK4 and cyclin D1: More than 10% of cancer cells expressed

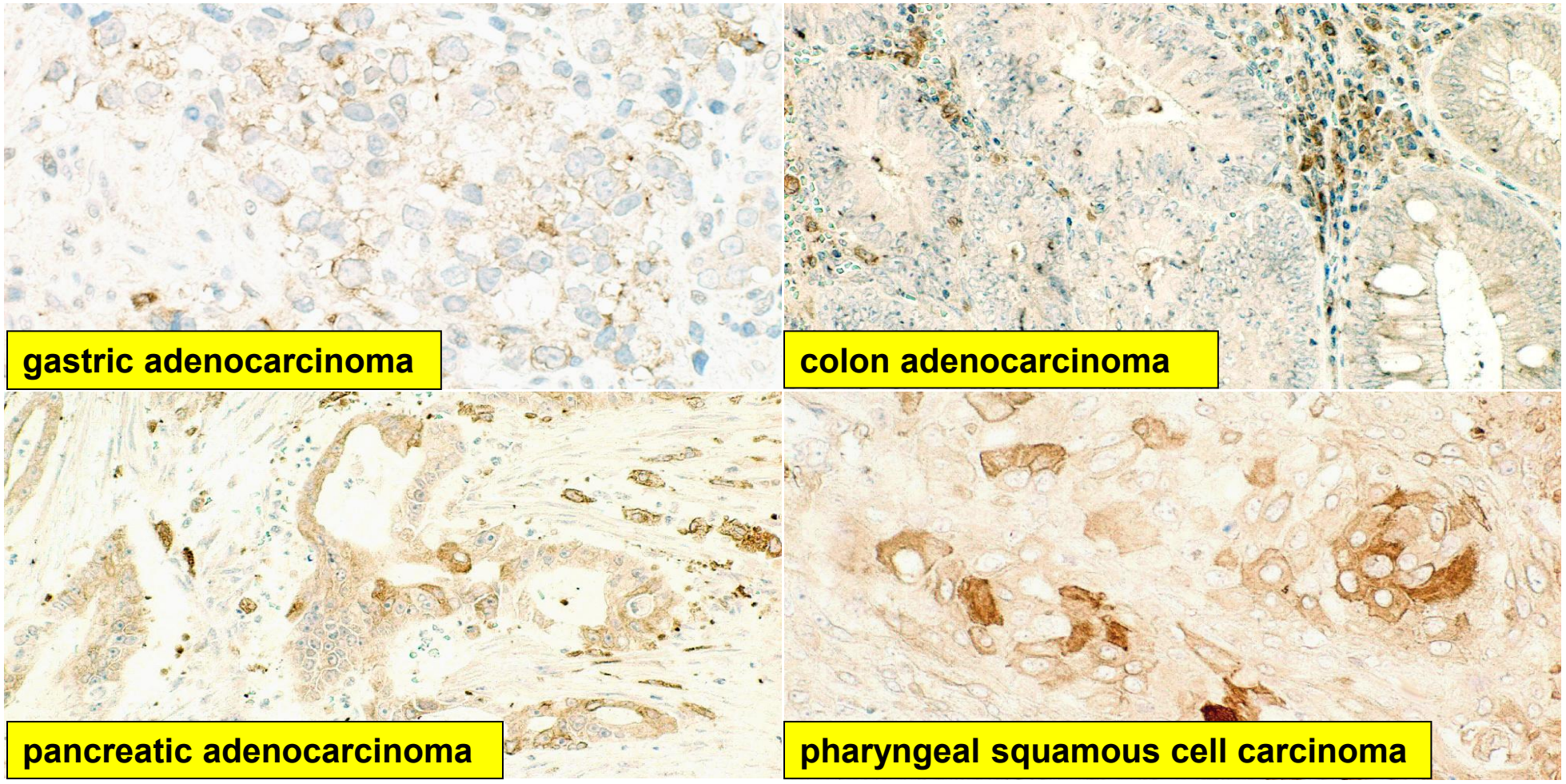
*p16^{INK4a}, CDK4 and cyclin D*1: "Nuclear positivity" was regarded as a criterion for high expressors.

Expression of thymidylate synthase (TS) in colorectal cancer and non-neoplastic mucosa



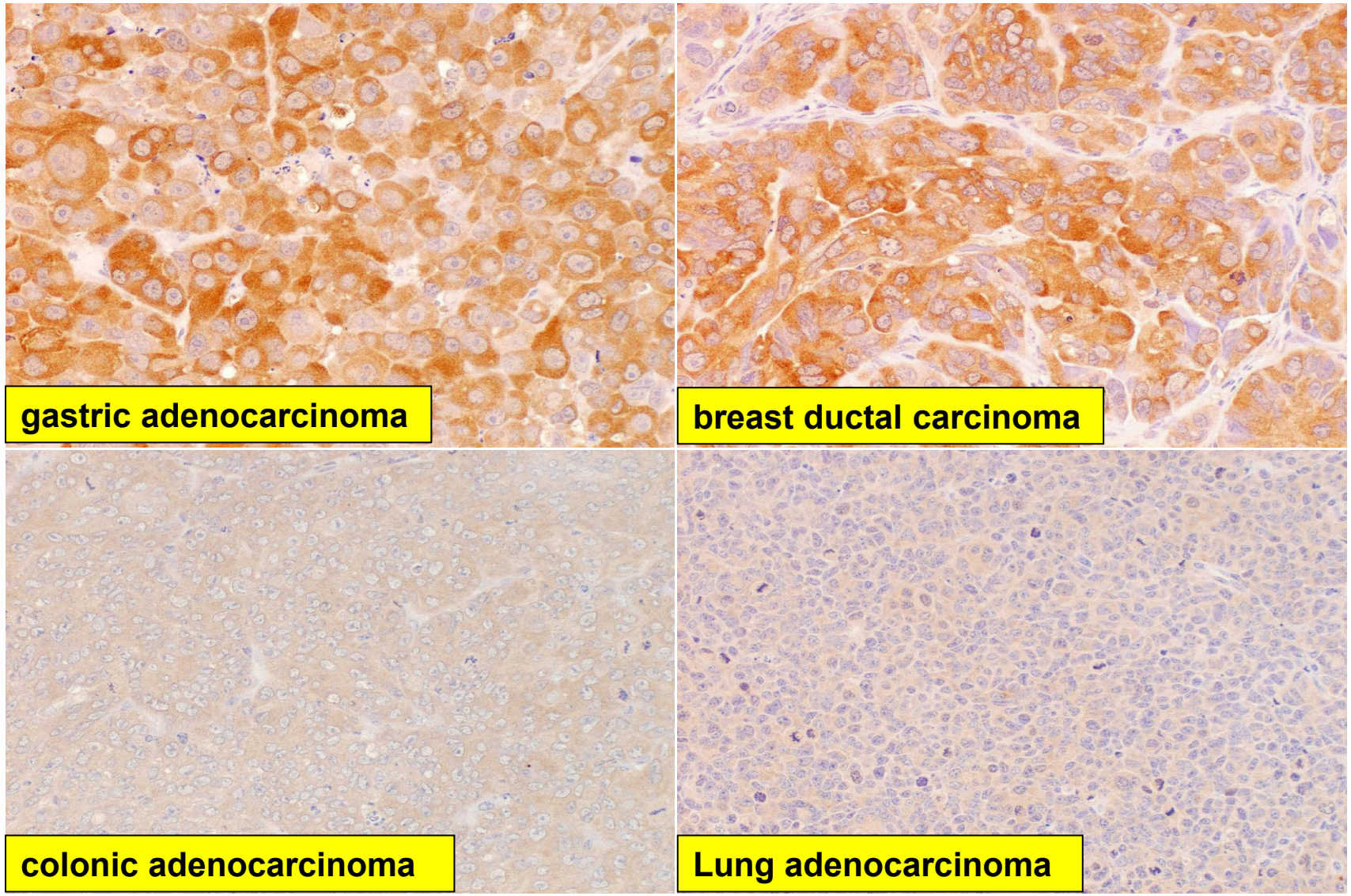
Of note is that TS expression in the normal colonic mucosa correlates with that of colorectal cancer.

Expression of DPD (dihydropyrimidine dehydrogenase) in varied adenocarcinomas (after heating in EDTA solution, pH 8)



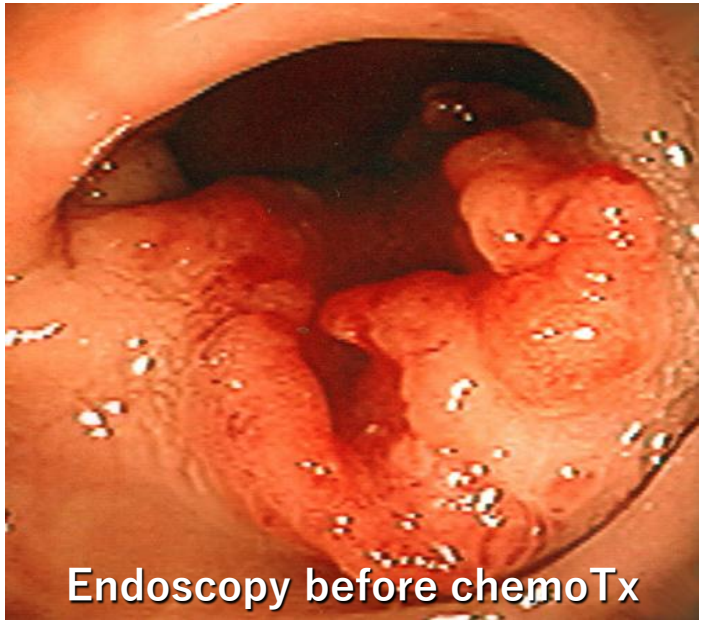
DPD is not expressed in colonic adenocarcinomas.

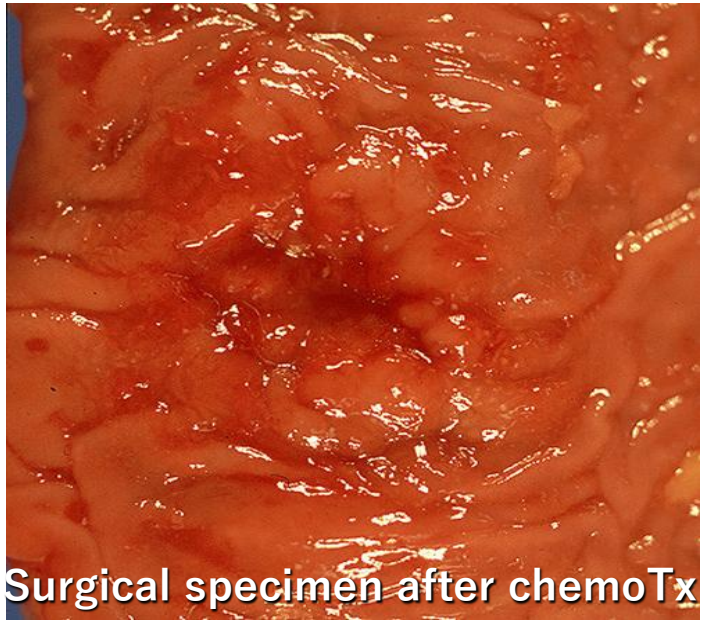
Expression of OPRT（orotate phosphoribosyltransferase）in varied adenocarcinomas



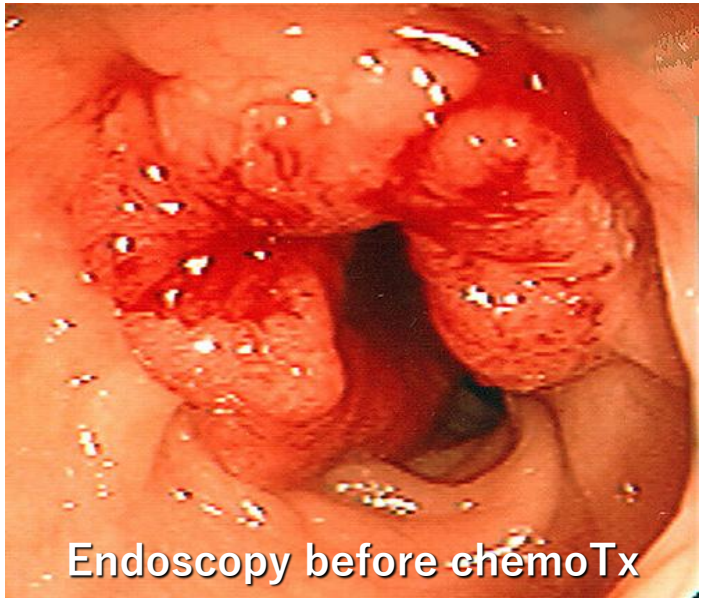
OPRT immunoreactivity is low in colonic adenocarcinomas.

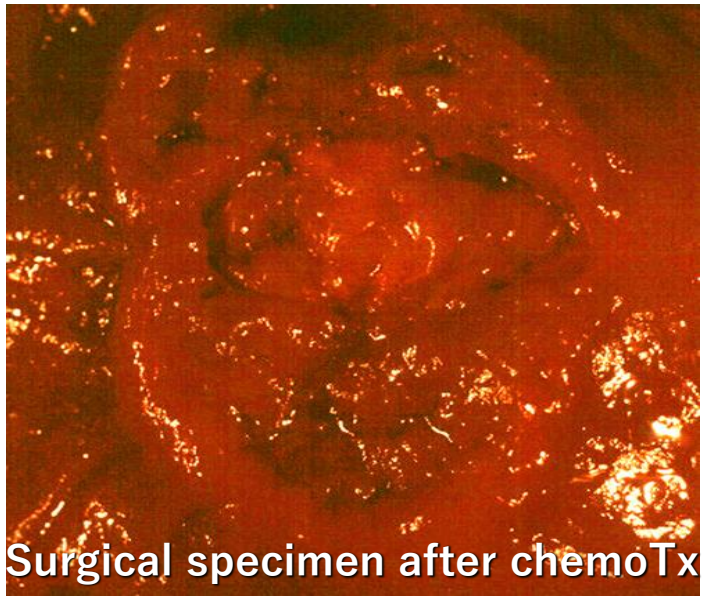
Case 1





Case 2





Clinical effects of preoperative chemotherapy:
(Two sigmoid colon cancers of Grade 2 responders)

Expression of TS, $p16^{INK4a}$, CDK4 and cyclin D1 in the surgical specimens and relationship to the effect of the preoperative chemotherapy

Protein expression	Responders (R) (%) n=6	Non-responders (NR) (%) n=31	Control lesions (C) (%) n=31	*p*-value R vs NR	*p*-value R vs C	*p*-value NR vs C
TS						
Low expression	6 (100)	8 (26)	12 (39)	0.001	0.008	0.42
High expression	0 (0)	23 (74)	19 (61)			
$p16^{INK4a}$						
Low expression	1 (17)	15 (48)	21 (68)	0.21	0.03	0.20
High expression	5 (83)	16 (52)	10 (32)			
CDK4						
Low expression	6 (100)†	28 (90)	25 (81)	>0.99	0.56	0.47
High expression	0 (0)	3 (10)	6 (19)			
Cyclin D1						
Low expression	6 (100)†	28 (90)	19 (61)	>0.99	0.15	0.02
High expression	0 (0)	3 (10)	12 (39)			

Responders (R): 6 cases （16%、 Grade 1b： 4 cases and Grade 2 : 2 cases）

Non-responders (NR): 31 cases （84% ; Grade 0 : 22 cases and Grade 1a: 9 cases）

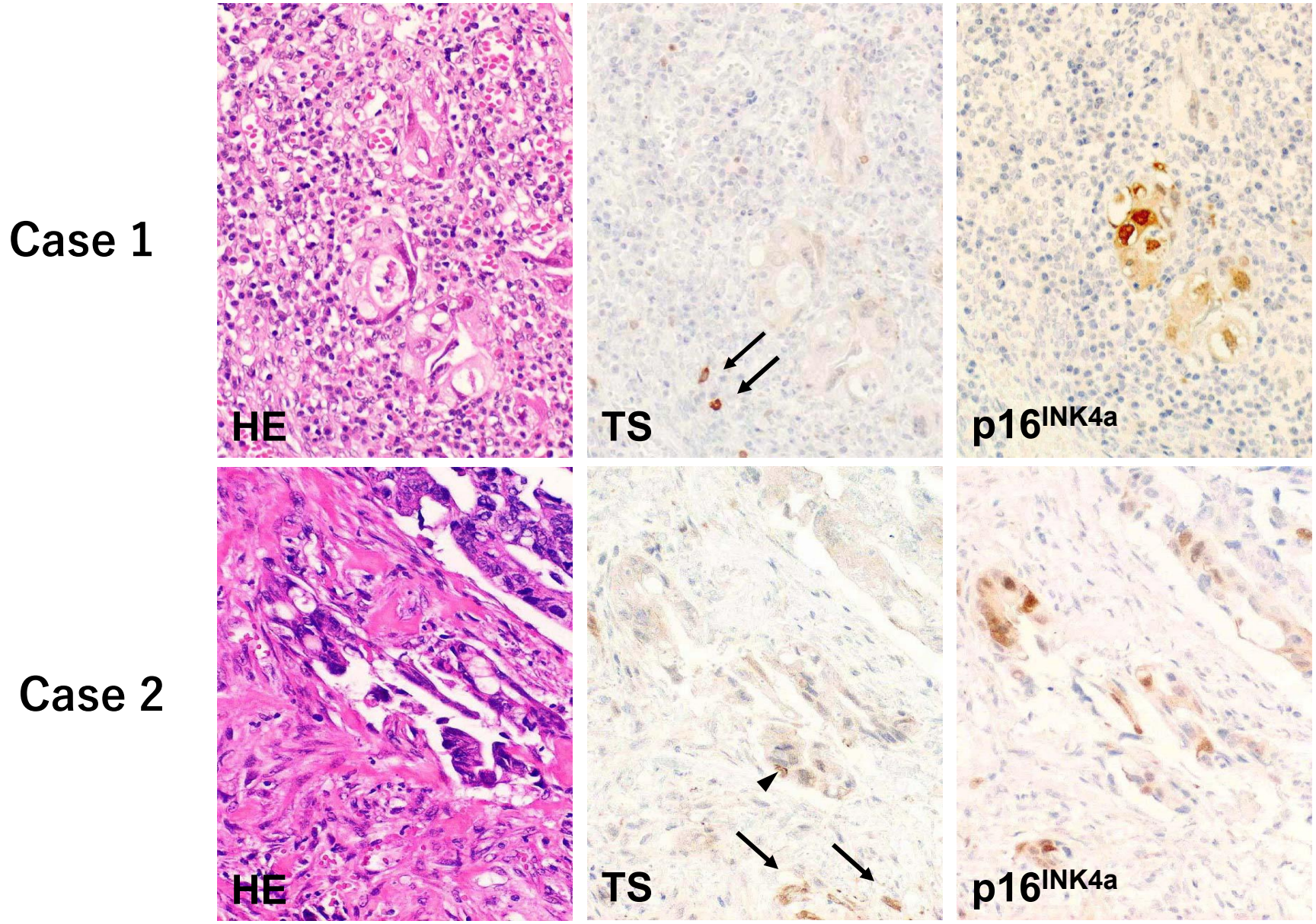
The responders showed low expression of TS and high expression of $p16^{INK4a}$.

The nonresponders showed low expression of cyclin D1.

Expression of $p16^{INK4a}$, CDK4 and cyclin D1 in TS-low and TS-high lesions, and the relationship with the effect of preoperative chemotherapy using 5-FU (analysis using the surgical material)

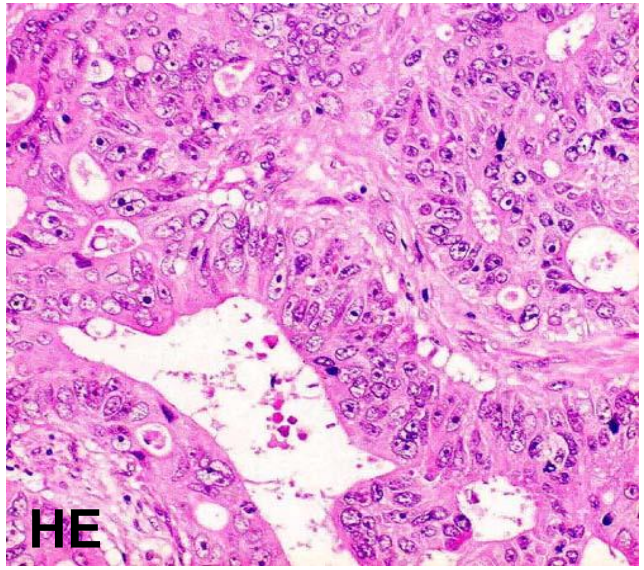
Protein expression	Responders (R) (%)	Non-responders (NR) (%)	Control lesions (C) (%)	*p*-value R vs NR	*p*-value R vs C	*p*-value NR vs C
TS-low	n=6	n=8	n=12			
p16-low	1 (17)	7 (88)	7 (58)	0.03	0.15	0.32
p16-high	5 (83)	1 (13)	5 (42)			
CDK4-low	6 (100)	7 (88)	10 (83)	>0.99	0.53	>0.99
CDK4-high	0 (0)	1 (13)	2 (17)			
cyclin D1-low	6 (100)	7 (88)	6 (50)	>0.99	0.05	0.16
cyclin D1-high	0 (0)	1 (13)	6 (50)			
TS-high	n=0	n=23	n=19	———	———	
p16-low	———	8 (35)	14 (74)	———	———	0.02
p16-high		15 (65)	5 (26)			
CDK4-low	———	21 (91)	15 (79)	———	———	0.38
CDK4-high		2 (9)	4 (21)			
cyclin D1-low	———	19 (83)	13 (68)	———	———	0.47
cyclin D1-high		4 (17)	6 (32)			
TS-low and p16 high	5 (83)	1 (3)	5 (16)	<0.0001	0.003	0.20
TS-low or p16 high	1 (17)	30 (97)	26 (84)			

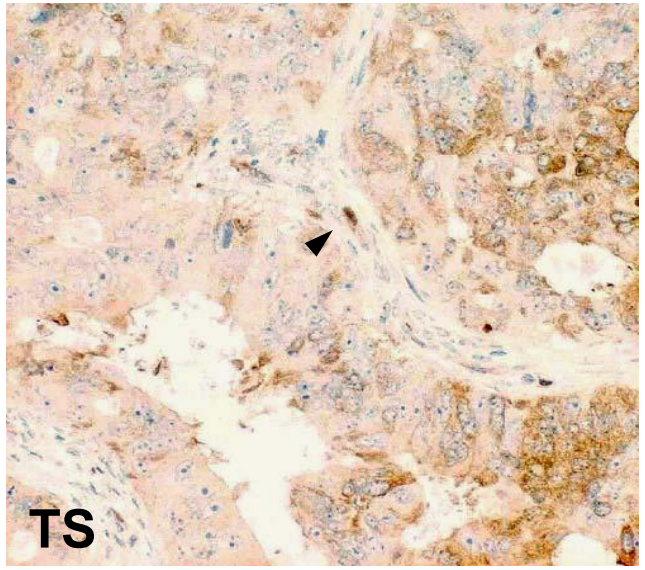
The responders significantly accompanied TS-low and $p16^{INK4a}$-high patterns.

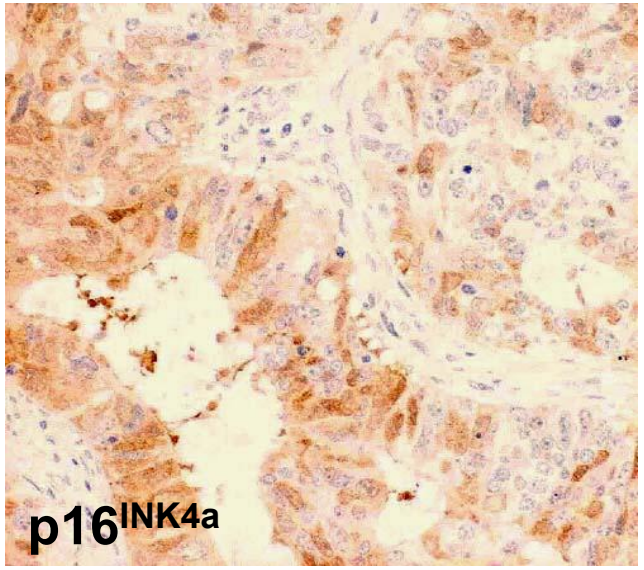


Expression of TS and $p16^{INK4a}$ in Grade 2 responders: 2 cases of sigmoid colon cancer (surgical specimens). Plasma cells in the stroma strongly express TS (arrows).

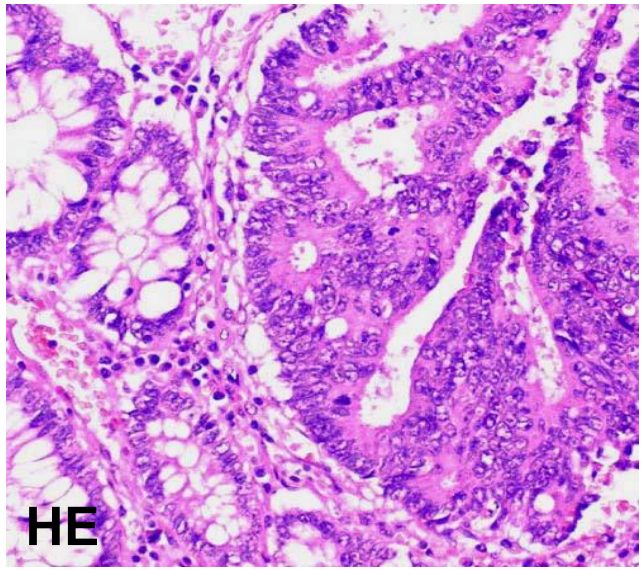
Case A

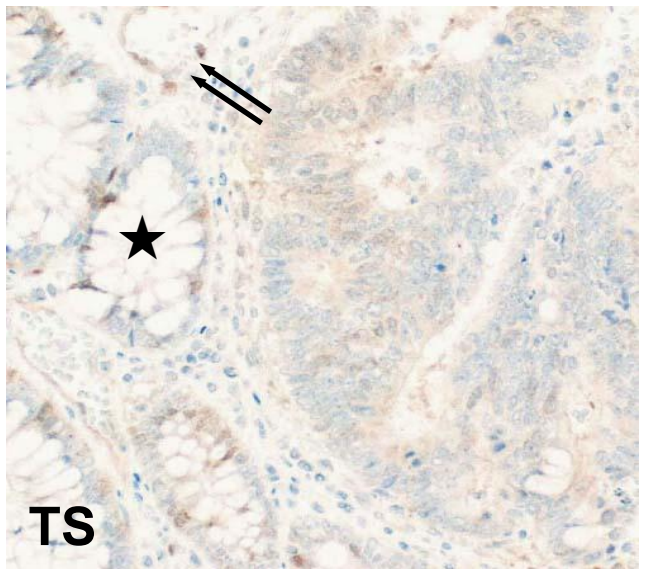


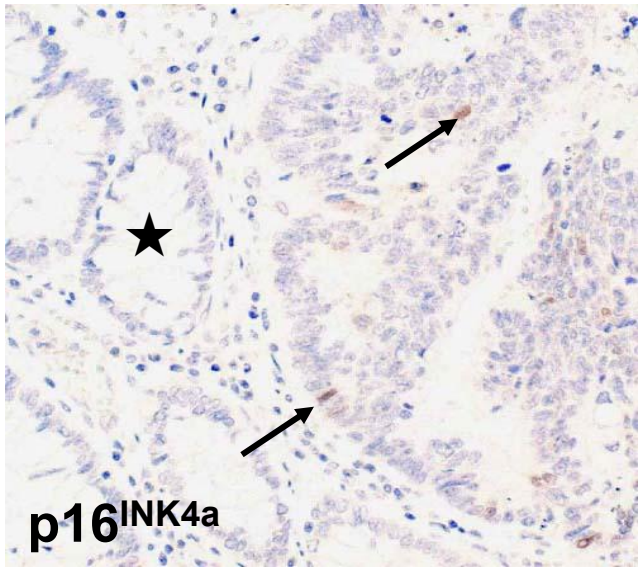




Case B



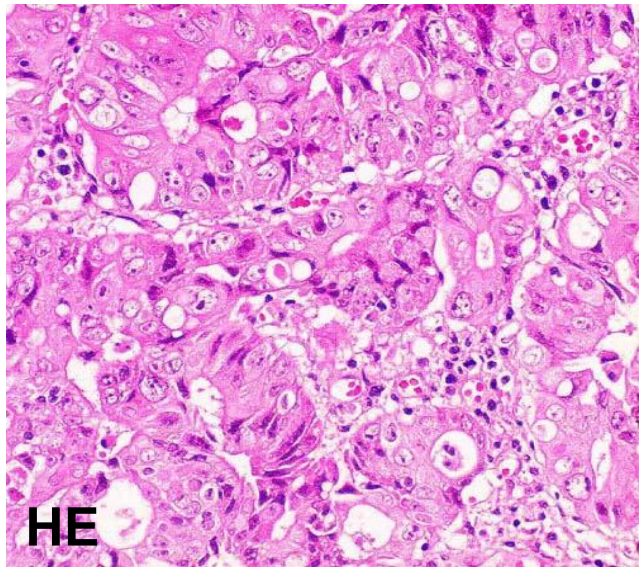


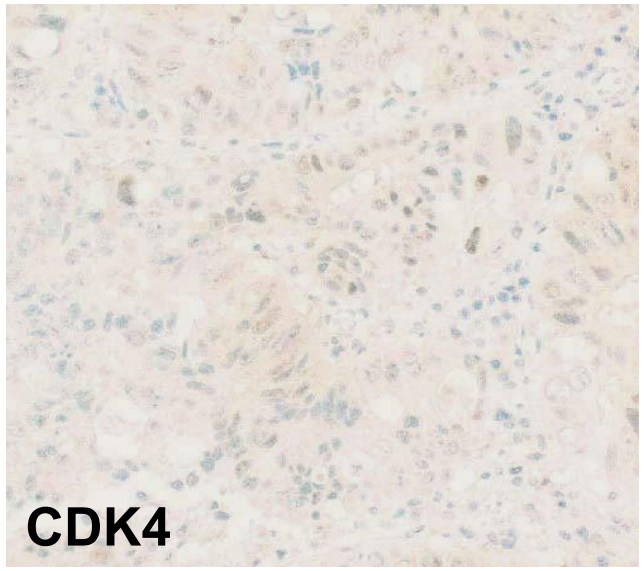


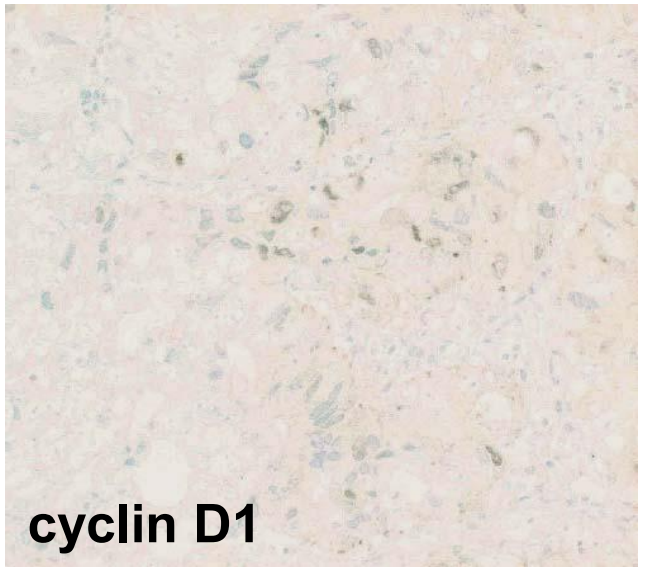
Asterisks:
normal crypts

Expression of TS and $p16^{INK4a}$ in non-responders: 2 cases of sigmoid colon cancer (surgical specimens). In case A expressing both TS and $p16^{INK4a}$, the expression pattern is reciprocal.

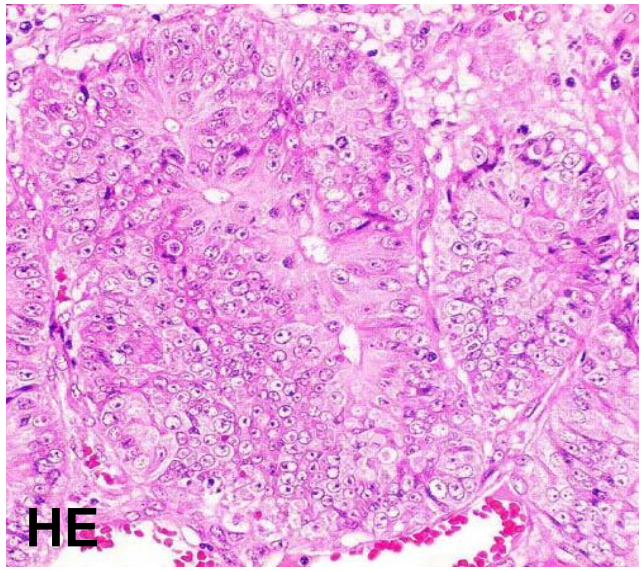
Non-responder (S. colon)

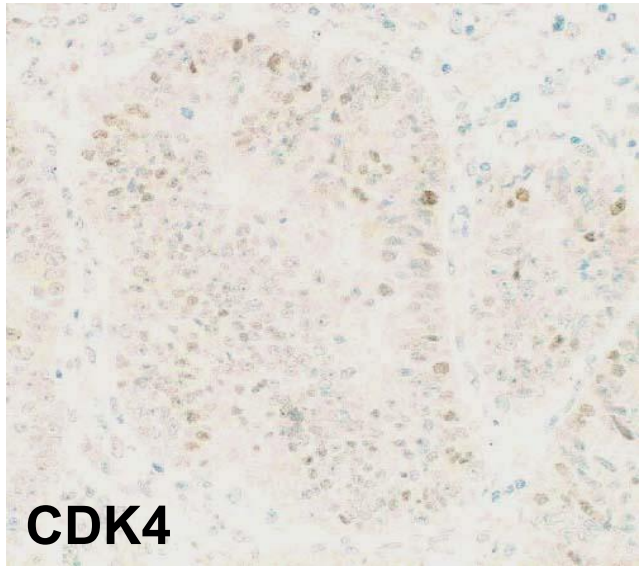


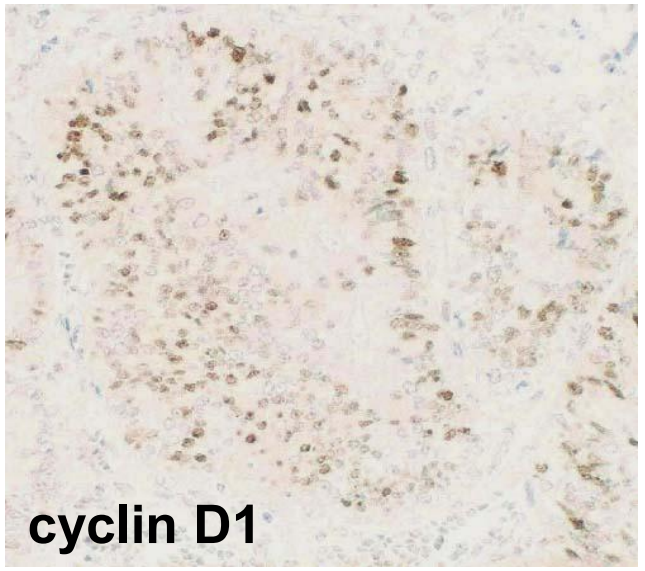




Control lesion (rectum)







Expression of CDK4 and cyclin D1 in a non-responder and a control lesion (surgical specimens). After administration of 5-FU, cyclin D1 expression is lowered.

Correlation of TS expression between preoperative biopsy specimens and surgical specimens: The significance of the number of biopsy specimens

Biopsy		Surgical specimen		Match
Numbers of Bx samples	TS expression	TS-low	TS-high	(%)
Preoperative chemoTx group (n=30)				
1 (n=5)	TS-low	1	1	2 (40)
	TS-high	2	1	
2 or more (n=25)	TS-low	8	3	20 (80)
	TS-high	2	12	
Control group (n=30)				
1 (n=7)	TS-low	0	2	4 (57)
	TS-high	1	4	
2 or more (n=18)	TS-low	6	0	15 (83)
	TS-high	3	9	

TS expression in colorectal cancer was comparable between the biopsy and surgical specimens, when the number of biopsy was two or more. The probability was 80% but without statistical significance.

Relationship between the chemotherapeutic effects and the expression of TS, $p16^{INK4a}$, CDK4 and cyclin D1 using preoperative biopsy specimens (when 2 or more biopsy specimens are available)

Protein expression	Responders (R) (%) n=5	Non-responders (NR) (%) n=20	Control group (C) (%) n=17	*p*-value R vs NR	*p*-value R vs C	*p*-value NR vs C
TS						
TS-low	5 (100)	7 (35)	5 (29)	0.01	0.01	>0.99
TS-high	0 (0)	13 (65)	12 (71)			
$p16^{INK4a}$						
p16-low	4 (80)	15 (75)	11 (65)	>0.99	>0.99	0.72
p16-high	1 (20)	5 (25)	6 (35)			
CDK4						
CDK4-low	4 (80)	19 (95)	15 (88)	0.37	>0.99	0.58
CDK4-high	1 (20)	1 (5)	2 (12)			
Cyclin D1						
cyclin D1-low	5 (100)	14 (70)	13 (76)	0.29	0.54	0.72
cyclin D1-high	0 (0)	6 (30)	4 (24)			

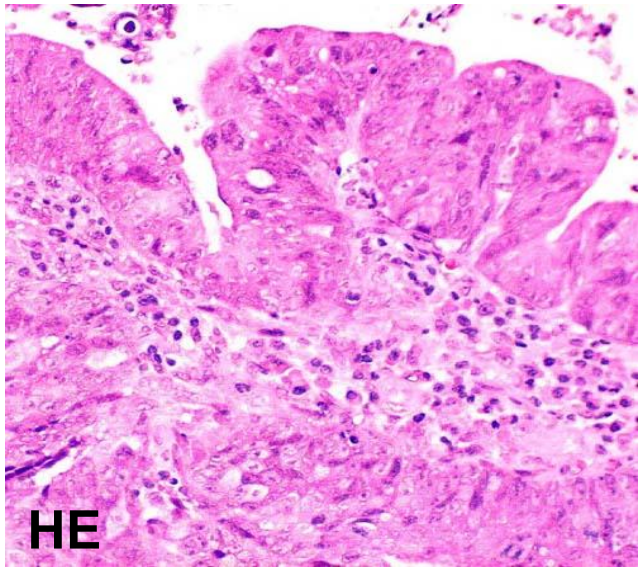
TS-low expression in preoperative biopsy specimens can predict the responders for preoperative chemotherapy. $p16^{INK4a}$, CDK4 and cyclin D1 give no specific correlation.

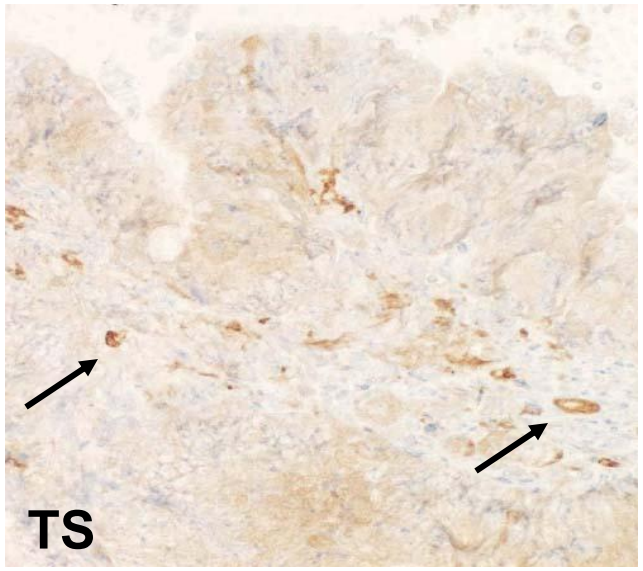
Correlation of the effect of preoperative chemotherapy and the expression of TS, $p16^{INK4a}$, CDK4 and cyclin D1 in the biopsy specimens (when 2 or more biopsy pieces are evaluated) and in the surgical specimens

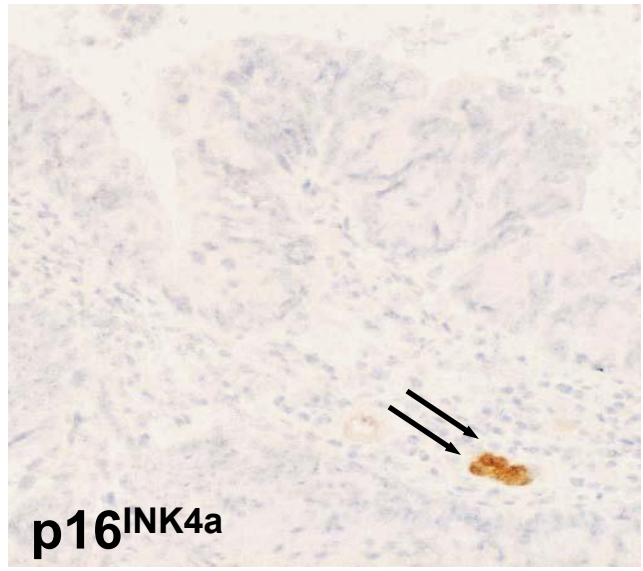
Protein expression	Responders (R) (%) n=5		Nonresponders (NR) (%) n=20		Control group (C) (%) n=17		*p*-value		
	Bx	Rx	Bx	Rx	Bx	Rx	R vs NR	R vs C	NR vs C
TS									
TS-low	5 (100)	5 (100)	7 (35)	5 (25)	5 (29)	8 (47)	0.59	0.91	0.23
TS-high	0 (0)	0 (0)	13 (65)	15 (75)	12 (71)	9 (53)			
$p16^{INK4a}$									
p16-low	4 (80)	1 (20)	15 (75)	10 (50)	11 (65)	12 (71)	0.27	0.01	0.09
p16-high	1 (20)	4 (80)	5 (25)	10 (50)	6 (35)	5 (29)			
CDK4	1 (20)	4 (80)	5 (25)	10 (50)					
CDK4-low	4 (80)	5 (100)	19 (95)	19 (95)	15 (88)	13 (76)	0.28	0.29	0.61
CDK4-high	1 (20)	0 (0)	1 (5)	1 (5)	2 (12)	4 (24)			
Cyclin D1									
cyclin D1-low	5 (100)	5 (100)	14 (70)	18 (90)	13 (76)	11 (65)	0.36	>0.99	0.11
cyclin D1-high	0 (0)	0 (0)	6 (30)	2 (10)	4 (24)	6 (35)			

In the responder group, the rate of $p16^{INK4a}$–high cases is significantly increased in the surgical specimen after 5-FU chemotherapy.

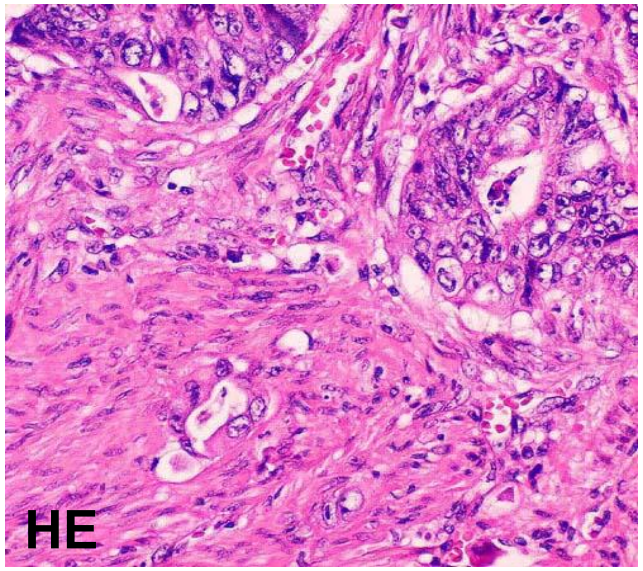
Biopsy

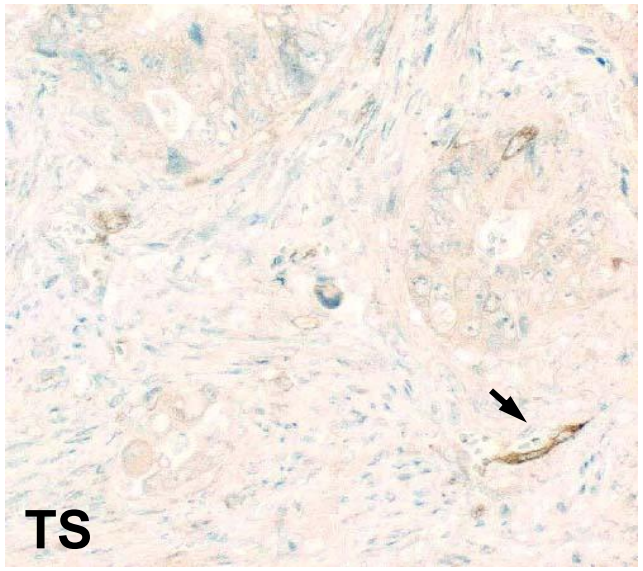


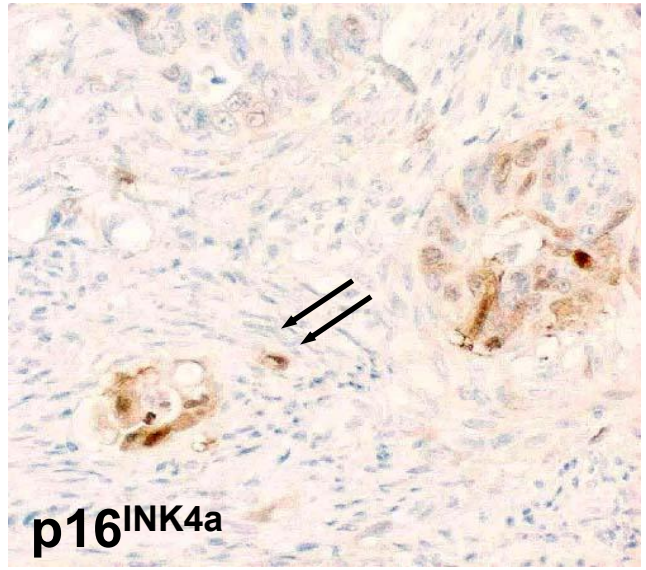




Surgical specimen







Comparative expression of TS and $p16^{INK4a}$ in preoperative biopsy and surgical specimens of Grade 2 responder of sigmoid colon cancer

Brief summary

1. TS-high lesions were not observed in the responder group. This indicates that the TS-high state is related to the 5-FU resistance of the cancer cells.

2. TS-low lesions were seen in the non-responder group. This indicates that 5-FU susceptibility can not be explained only by the TS-low state.

3. Responders predominantly showed TS-low and $p16^{INK4a}$-high patterns. This suggests that the 5-FU susceptibility requires both the TS-low state and the cell cycle arrest by $p16^{INK4a}$ expression.

4. In the responder group, the expression of $p16^{INK4a}$ was often induced after preoperative chemotherapy. This suggests that the induction of $p16^{INK4a}$ may plays an important role in the 5-FU susceptibility of colorectal cancer.

5. TS and $p16^{INK4a}$ were reciprocally expressed in the colorectal cancer. This suggests that the TS-high state may inhibit the cell cycle arrest induced by $p16^{INK4a}$.

6. TS expression was well correlated between the preoperative biopsy specimens (when two or more pieces were biopsied) and the surgical specimens. This is practically important for us to predict immunohistochemically the 5-FU resistance or susceptibility of colorectal cancer.

7. There was no correlation between the chemotherapeutic effect and the expression of CDK4 and cyclin D1.

Prognosis of the patients with colorectal cancer after 5-FU-based preoperative chemotherapy and the immunohistochemical expression of TS and $p16^{INK4a}$

Patients: 132 cases of colorectal cancer after 5-FU-based preoperative chemotherapy

Immunostainiong for TS and $p16^{INK4a}$: Use of formalin-fixed, paraffin-embedded sections

Follow-up period: 64 months on average

Survival period: evaluable in 113 cases

1) Short term survival (death within 5 years)

2) Long term survival with recurrence (alive for 5 years or more, but with recurrent tumors)

3) Long term survival without recurrence (alive for 5 years or more without recurrence)

Clinicopathological factors and the expression of TS and p16^{INK4a} in colorectal cancer after preoperative chemotherapy

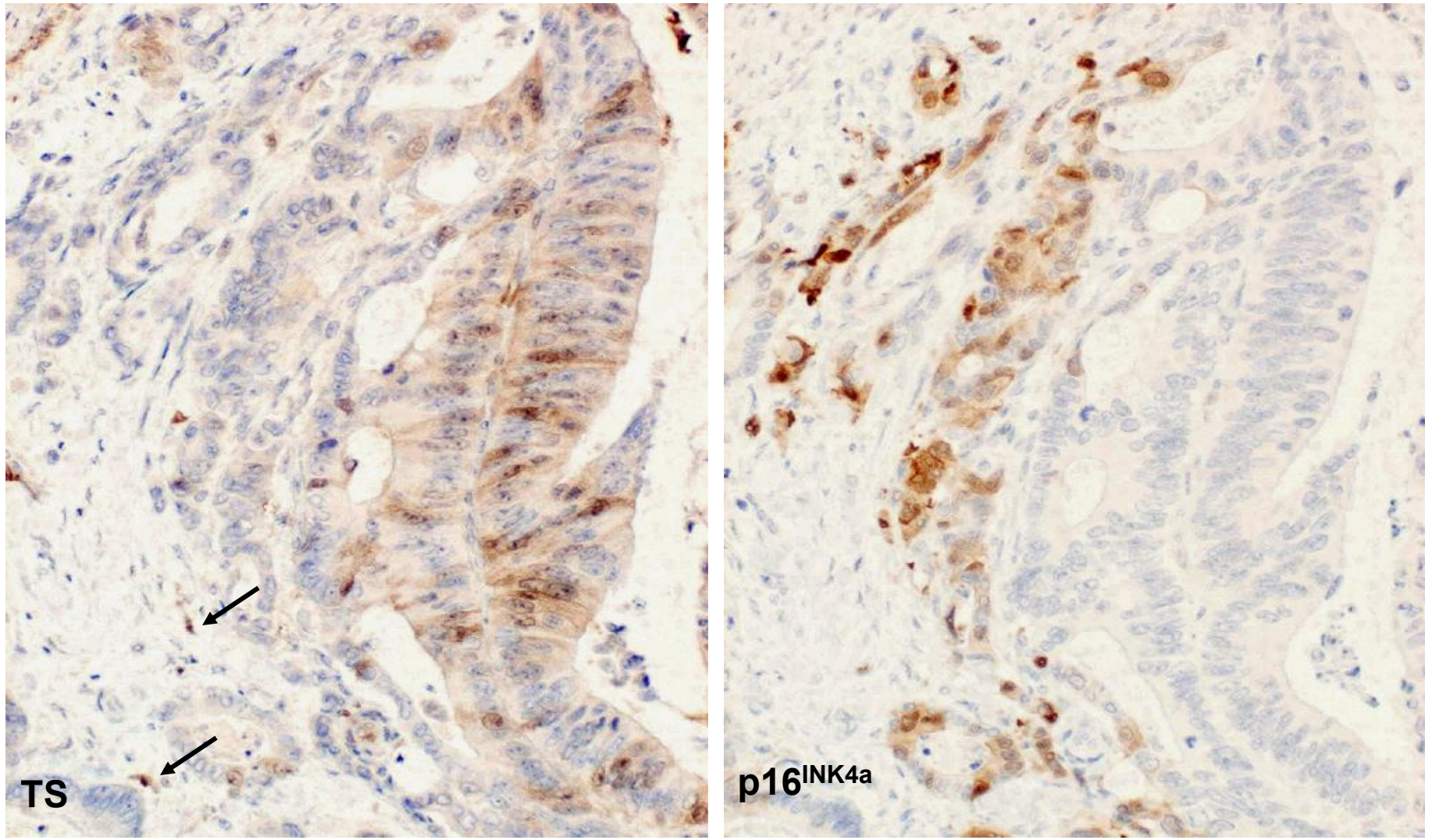
Clinicopathological factor	TS			p16^{INK4a}		
	TS-low	TS-high	*p*-value	p16-low	p16-high	*p*-value
Age						
<65	51	17		48	20	
≥65	39	25		39	25	
Gender						
men	55	25		52	28	
women	35	17		35	17	
Primary site						
Colon	35	15		34	16	
Rectum	55	27		53	29	
Differentiation						
G1	41	16		42	15	
G2	48	26		44	30	
Depth of invasion						
T2/T3	48	25		48	25	
T4	42	17		39	20	
Nodal met						
N0 (no met)	35	8		29	14	
N1 (1-3 nodes)	37	14	0.03	36	15	0.002
N2 (≥4 nodes)	18	20		22	16	
Recurrence						
Simultaneous	16	20		22	14	
Allochronic	28	14		25	17	
Liver	27	24		33	18	
Lung	12	3		8	7	
Local	3	3		4	2	
Others	2	4		2	4	
Dukes' classification						
B	30	6		26	10	
C	44	16	0.008	39	21	0.001
D	16	20		22	14	
Regimen						
5-FU then UFT	63	31		61	33	
UFT alone	11	4		11	4	
5-FU alone	9	0		6	3	
Others	7	7		9	5	

The rate of TS-high lesions was significantly high in the N2 group and Dukes' D group. No correlation was detected for p16^{INK4a} expression.

Expression of TS and $p16^{INK4a}$ and the recurrence of colorectal cancer after 5-FU-based preoperative chemotherapy

Prognosis	TS			$p16^{INK4a}$		
	TS-low	TS-high	*p*-value	p16-low	p16-high	*p*-value
Dukes' B						
Recurrence (n=9)	8	1		6	3	
Non-recurrence (n=27)	22	5		20	7	
Dukes' C						
Recurrence (n=32)	19	13	0.02	19	13	
Non-recurrence (n=28)	25	3		20	8	
Dukes' D (n=36)	16	20		22	14	

In Dukes' C group, the rate of TG-high lesions was higher in the recurrence cases than the non-recurrence cases. However, in Dukes' B group, there was no correlation between the TS expression and recurrence. Regarding the expression of $p16^{INK4a}$, no correlation was observed between the recurrence and non-recurrence groups.

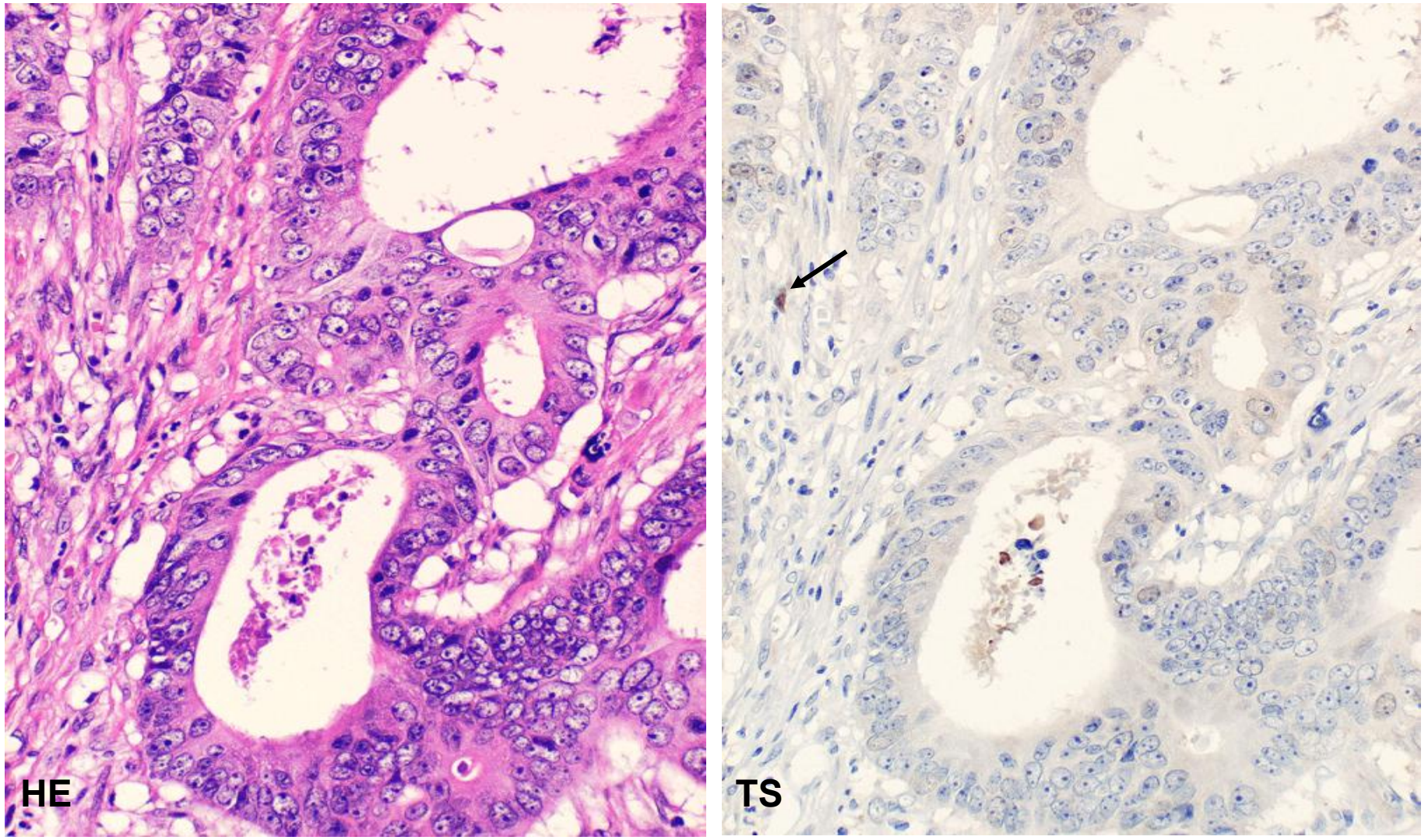


Expression of TS and $p16^{INK4a}$ in Dukes' C rectal cancer accompanying the liver recurrence 10 months after surgical removal. TS and $p16^{INK4a}$ are expressed reciprocally. Arrows indicate TS expression in plasma cells.

Correlation between the expression of TS and $p16^{INK4a}$ and the prognosis of the patients with colorectal cancer after 5-FU-based preoperative chemotherapy

Prognosis	TS			$p16^{INK4a}$		
	TS-low	TS-high	*p*-value	p16-low	p16-high	*p*-value
Short term survival (n=39)	25	14	0.01	21	18	
Long survival with recurrence (n=19)	12	7	0.04	14	5	
Long survival without recurrence (n=55)	48	7		40	15	

In the group with long term survival without recurrence, a significantly low rate of TS-high expression was observed. There is no correlation between the expression of $p16^{INK4a}$ and the survival rates.



TS expression in Dukes’ C rectal cancer showing long term survival without recurrence

Discussion

Previous studies so far failed to detect the value of immunohistochemical expression of TS in colorectal cancer.

The main reason for the discrepancy is the method of immunohistochemical staining applied. Appropriate application of the heat-induced epitope retrieval can significantly improve the reliability of TS immunostaining.

In Dukes' D group and lymph node N2 group, TS-high lesions are significantly frequent. The TS-high state may suggest a high metastatic potential of colorectal cancer.

Conclusions

1) Immunohistochemical observation of the TS-high status can predict the 5-FU resistance of colorectal cancer. Preoperative biopsy specimens can be employed for the prediction.

2) The TS-high state has a correlation with the patient poor prognosis.

3) The TS-low state and 5-FU-induced expression of $p16^{INK4a}$ can be an indicator of 5-FU susceptibility.