Apoproliferative cells in colon cancer induced by cytotoxic agents: cells expressing both apoptosis and cell-proliferation markers

1) Cells simultaneously expressing apoptotic and proliferation markers ("apoproliferative cells") can be demonstrated histochemically.
2) "Apoproliferative cells" appeared in correlation with cleaved caspase 3-positive apoptotic cells.
3) "Apoproliferative cells" in colon cancer tissue showed a peak number shortly (1-4 days) after 5-FU administration.
4) Proliferative activity was evidently suppressed when the "apoproliferative cells" showed a peak incidence.

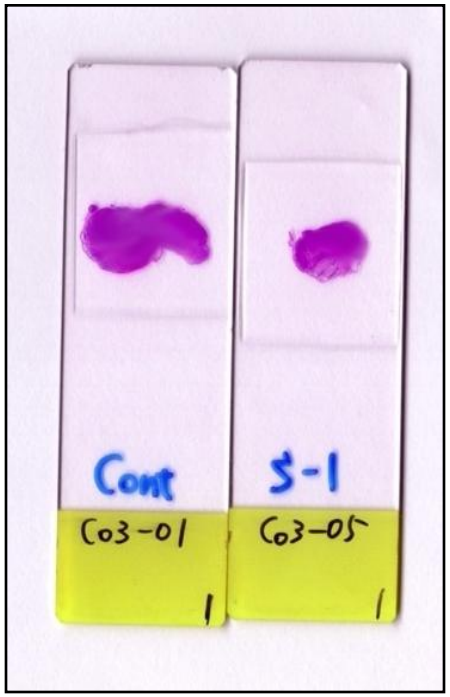
***Presented in 2010 by Yutaka Tsutsumi, Masayuki Ito, Yukako Iitani, Kazuya Shiogama and Shingo Kamoshida (Department of Pathology, Fujita Health University School of Medicine, Toyoake, Japan)**

Materials

・Nude mouse xenografts of human colon cancer cell lines（Co-3、COL-1、HCT15）and human gastric cancer cell lines（4-1ST, AZ- 521,SC-2,SC-4,St-40）

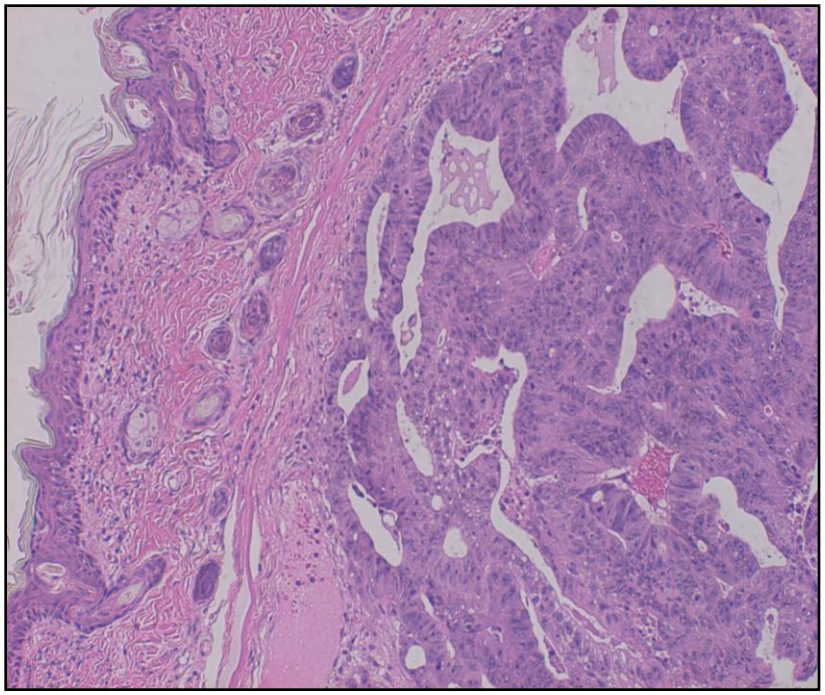
・Fluoropyrimidine anti-cancer drugs：UFT、S-1、5'-DFUR、capecitabine

・Formalin-fixed, paraffin-embedded sections sampled from tumor-laden mice after oral administration of anti-cancer drugs for 1 to 14 days



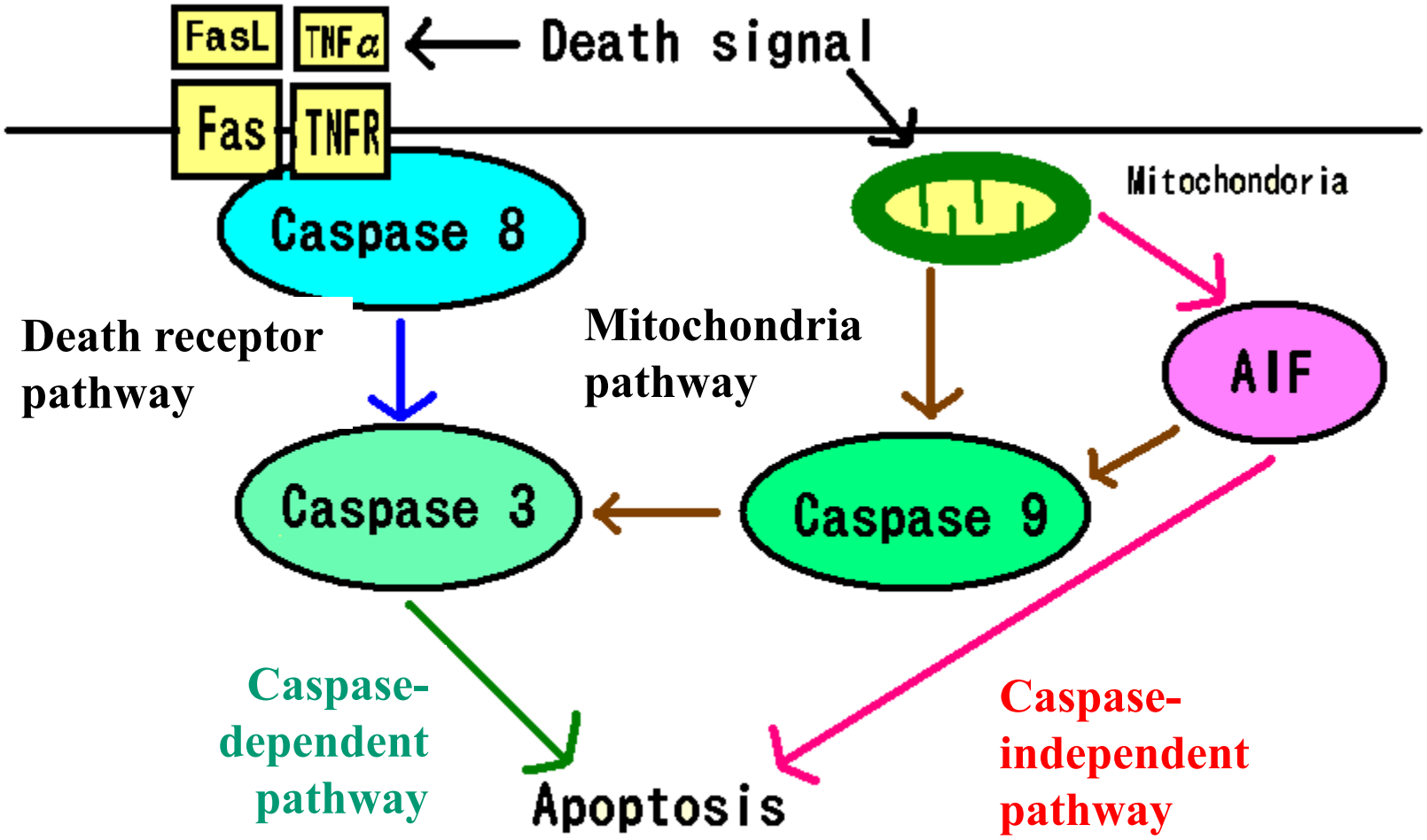
Control tumor (H&E)

After administration of S-1 (H&E)



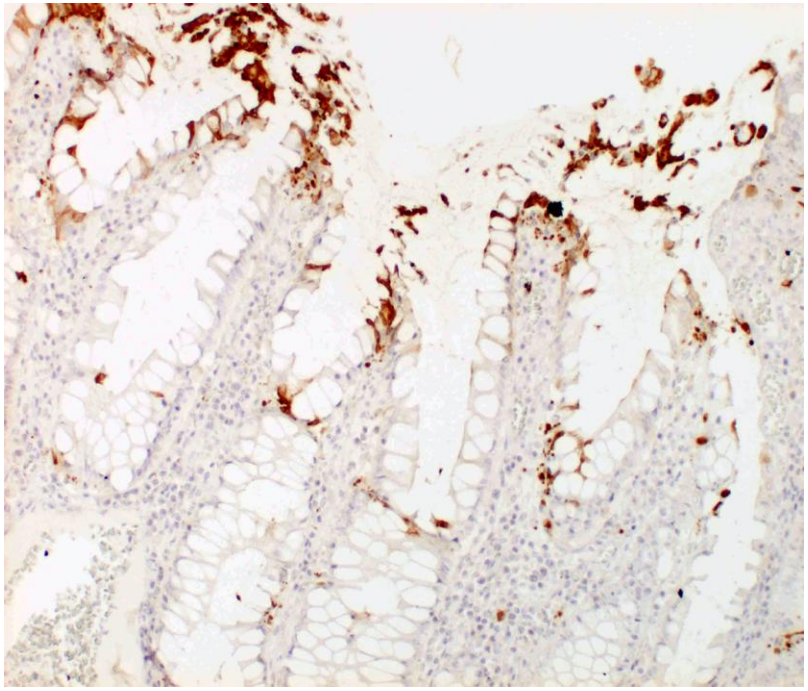
Xenograft of human colon cancer cell line Co-3 (H&E)

Caspase-dependent and independent cascades of apoptosis



AIF: apoptosis-inducing factor

Positive controls



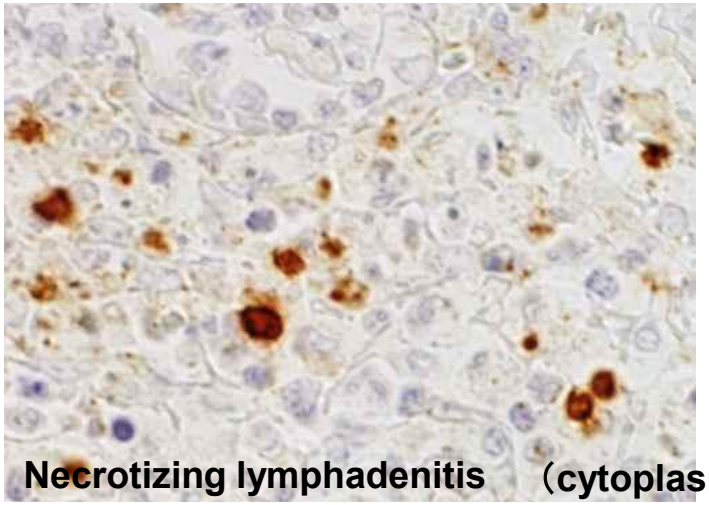
Normal human colon

Cleaved-caspase 3

pan-apoptosis marker

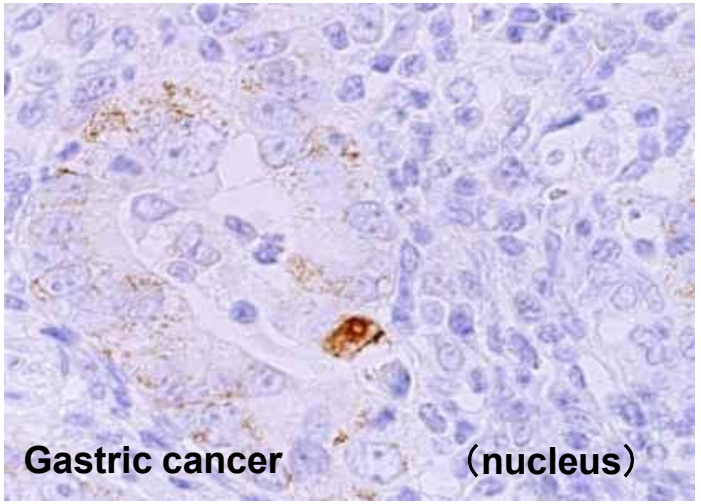
（positive in the cytoplasm）

Apoptosis is seen in the most superficial part of the colonic mucosa.



Cleaved caspase 9

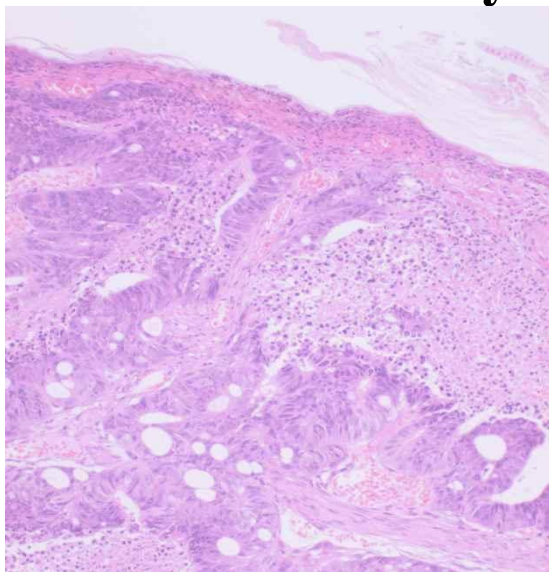
Apoptosis marker in the mitochondria pathway



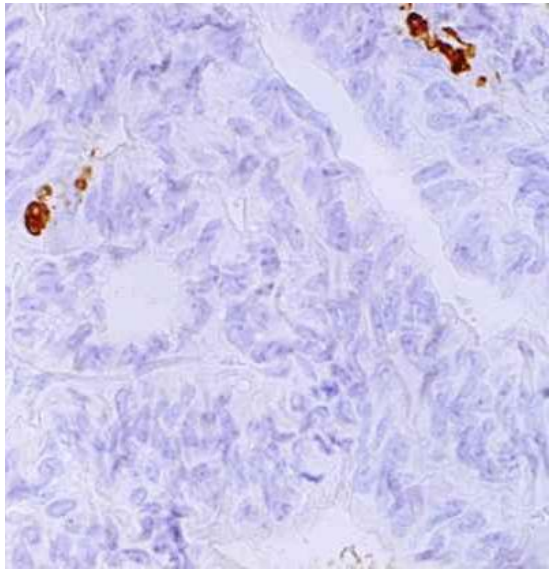
AIF (apoptosis-inducing factor)

Caspase-independent marker of apoptosis

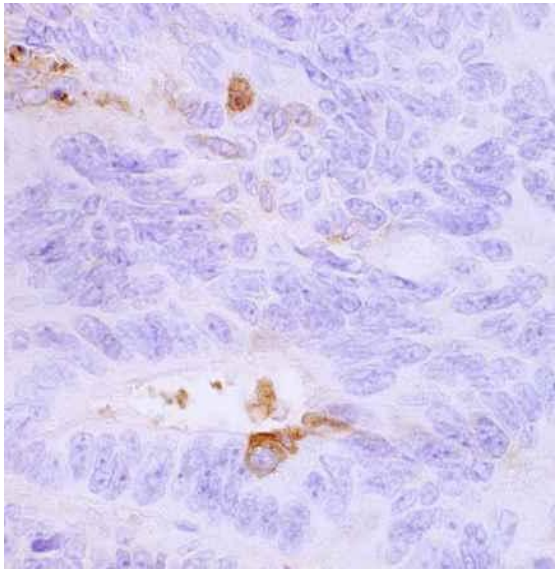
Apoptosis in colon cancer cell line (Co-3) zenograft induced by 5'-DFUR administration



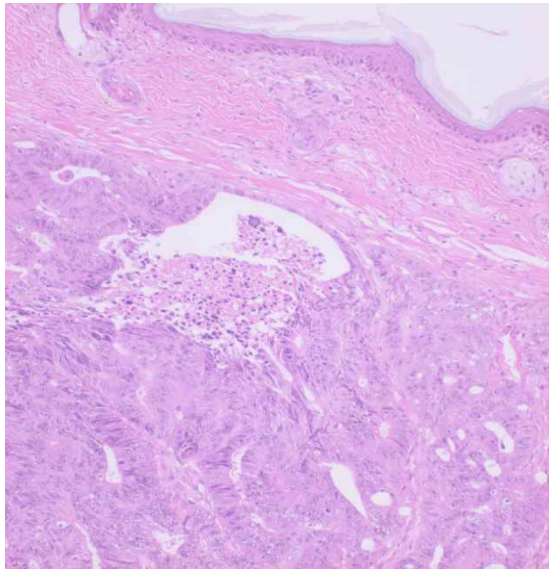
Control HE



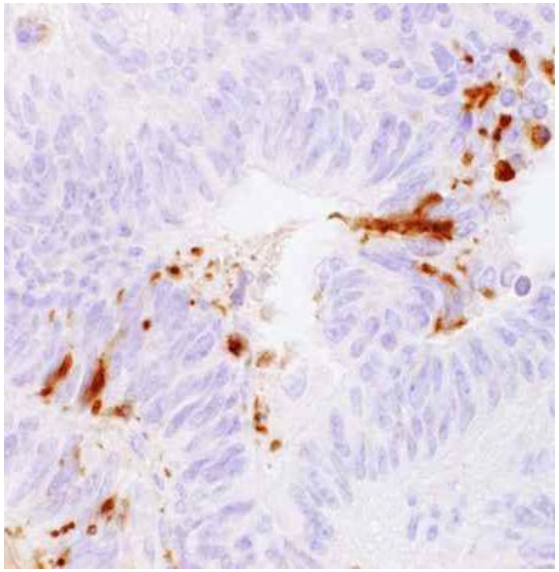
Control C-caspase 3



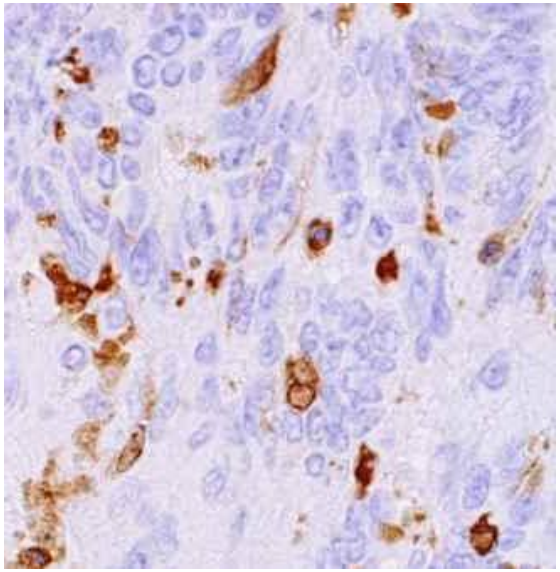
Control C-caspase 8



5'-DFUR administered HE

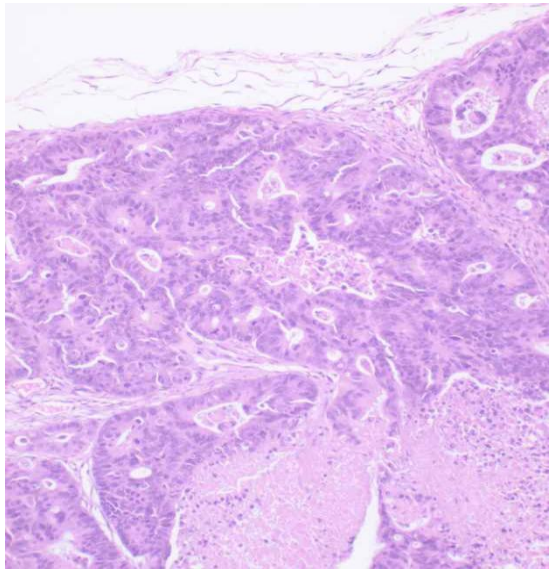


5'-DFUR administered C-caspase 3

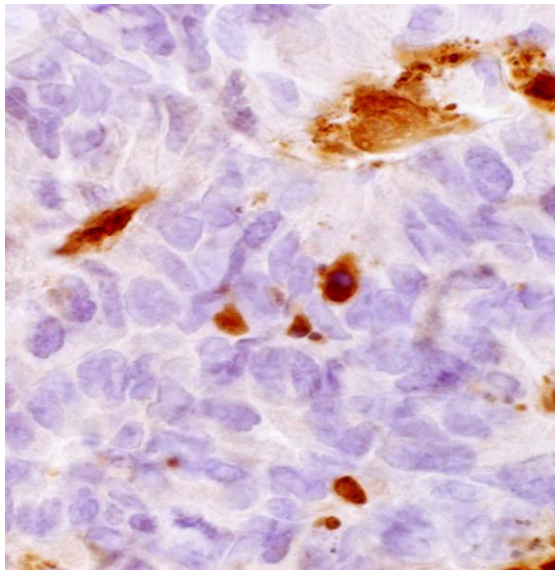


5'-DFUR administered C-caspase 8

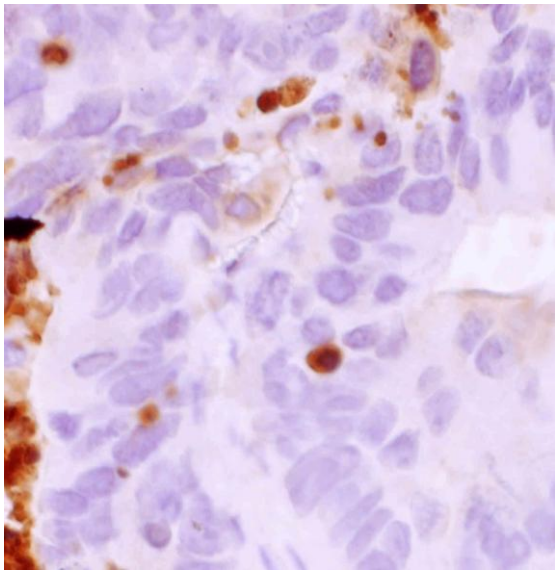
Apoptosis in gastric cancer cell line (St-40) zenograft induced by 5’-DFUR administration



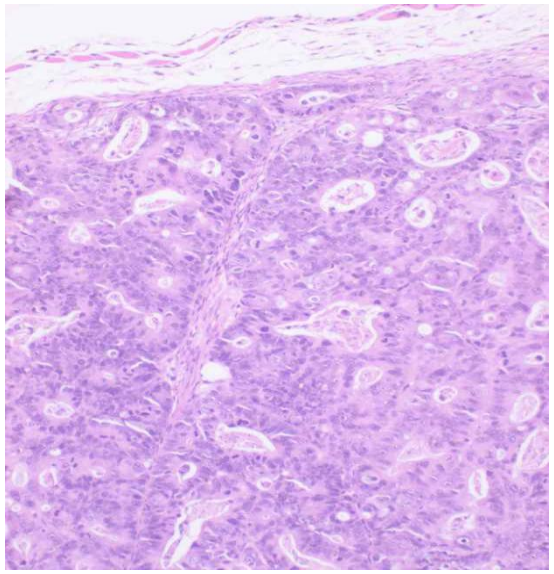
Control HE



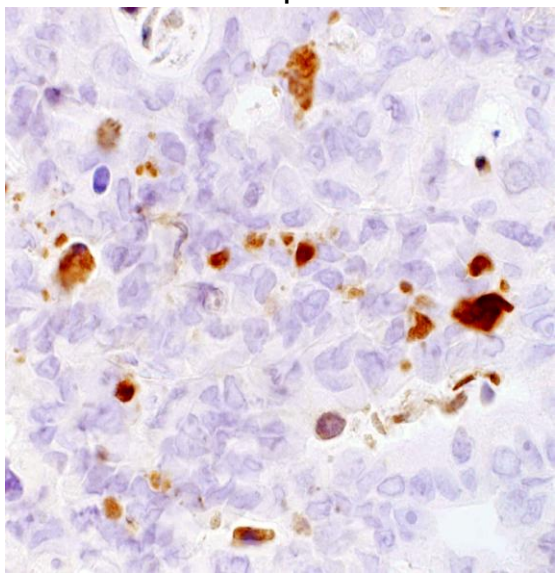
Control C-caspase 3



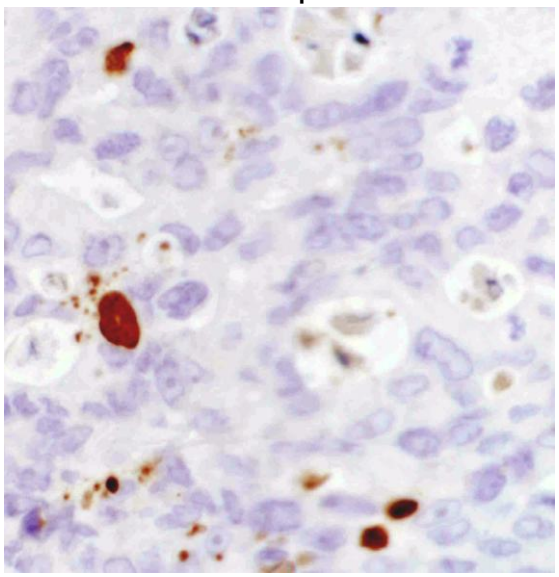
Control C-caspase 8



5’-DFUR administered HE

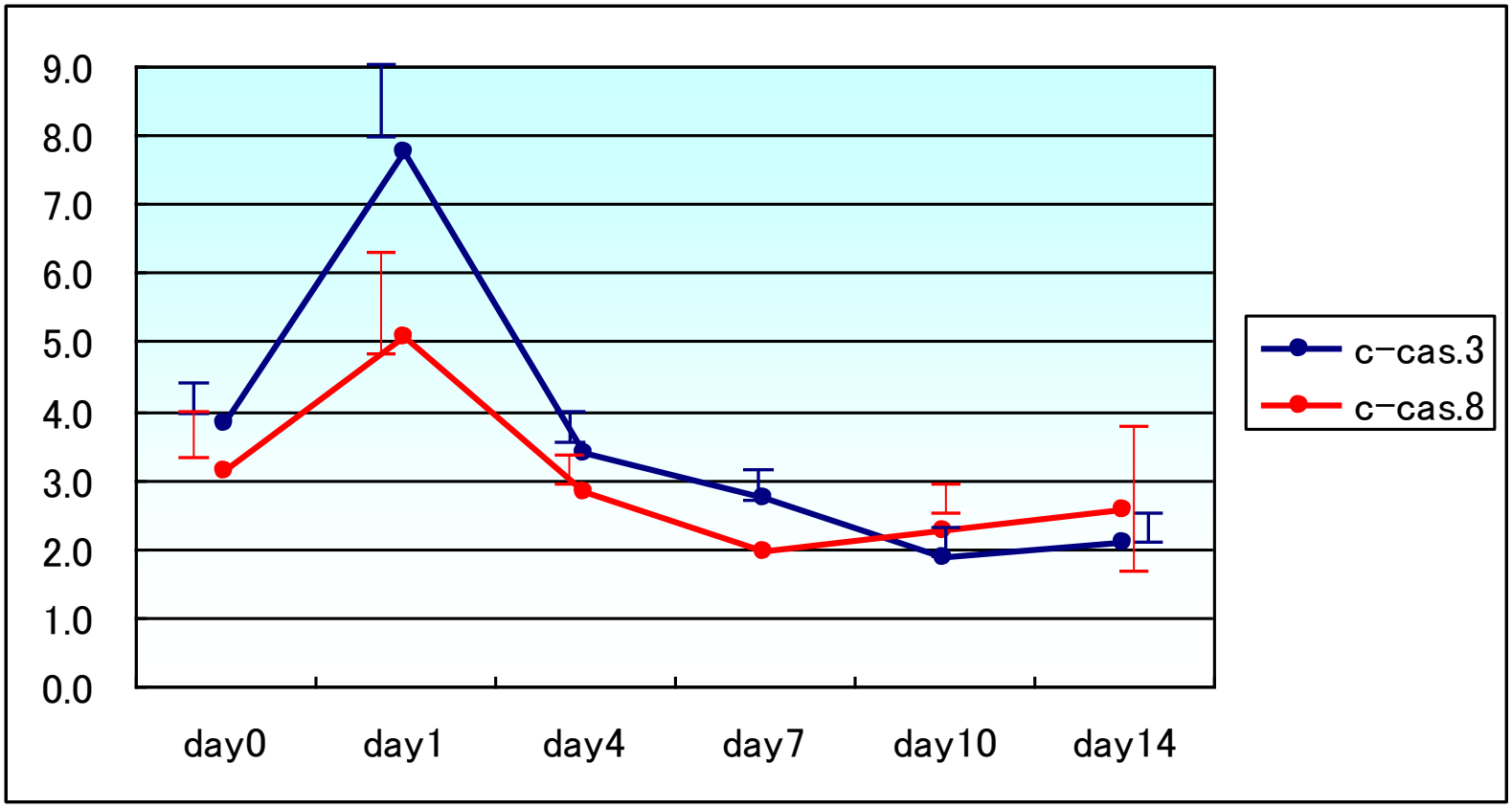


5’-DFUR administered C-caspase 3



5’-DFUR administered C-caspase 8

Colon cancer cell line COL-1
Change of apoptosis marker positive rates after administration of S-1



Total cell No.	182	200	210	181	128	130

Mean values of positive cells evaluated in microphotographs

Apoptosis in colon cancer cell lines induced by anti-cancer drugs

（oral administration for 14 days）

	Xenograft	TGI(%)	Positive rate（%）			
			C-caspase 3	C-caspase 8	C-caspase 9	AIF
Co-3	Control		2.0	1.0	~0	0
	UFT-treated	43	2.5	1.9	~0	0
	S-1-treated	62	2.7	1.9	~0	0
	5'-DFUR-treated	42	3.0	2.9	~0	0
	Capecitabine-treated	37	3.0	2.1	~0	0
COL-1	Control		2.4	2.8	~0	0
	UFT-treated	53	2.9	0.8	~0	0
	S-1-treated	74	2.4	2.4	~0	0
	5'-DFUR-treated	35	4.1	1.1	~0	0
	Capecitabine-treated	35	3.0	2.7	~0	0
HCT-15	Control		2.2	0.2	~0	0
	UFT-treated	46	3.4	0.7	~0	0
	S-1-treated	29	1.5	0.8	~0	0
	5'-DFUR-treated	48	1.2	0.6	~0	0

1335 cells evaluated on average

-------More cells were labeled than the controls

Apoptosis in gastric cancer cell lines induced by anti-cancer drugs (oral administration for 14 days)

	Xenograft	TGI(%)	Positive rate（%）			
			C-caspase 3	C-caspase 8	C-caspase 9	AIF
4-1ST	Control		3.4	2.5	0.4	0
	UFT-treated	33	3.9	4.9	1.7	0
	S-1-treated	43	6.1	4.1	1.4	0
	5’-DFUR-treated	28	7.2	4.9	1.1	0.9
AZ-521	Control		4.6	2.5	~0	0
	UFT-treated	34	5.6	2.9	~0	0.4
	S-1-treated	48	1.6	3.2	~0	0
	5’-DFUR-treated	26	3.4	3.0	~0	0
SC-2	Control		4.8	3.4	1.4	0
	UFT-treated	52	4.0	4.3	2.9	0
	S-1-treated	68	4.8	4.7	4.3	0
	5’-DFUR-treated	51	4.8	3.4	1.6	0

Summary

1) Cells positive for cleaved caspase 3 well correlated with cells positive for cleaved caspase 8.
2) 5-FU-induced apoptosis in colon cancer cells is mediated by the death receptor pathway with active cleavage of caspase 8.
3) Cleaved caspase 9 and AIF are negative in colon cancer.
4) 5-FU-induced apoptosis in gastric cancer is variegated: Some through the death receptor pathway, and others through the mitochondrial pathway. AIF is also focally expressed in gastric cancer cells.
5) The apoptosis in colon cancer showed a peak 1 to 4 days after 5-FU administration.

Detection of "apoproliferative cells"

"Apoproliferative cells" are defined as cells simultaneously showing cell proliferation and apoptotic markers.

・Double immunostaining with amino acid polymers

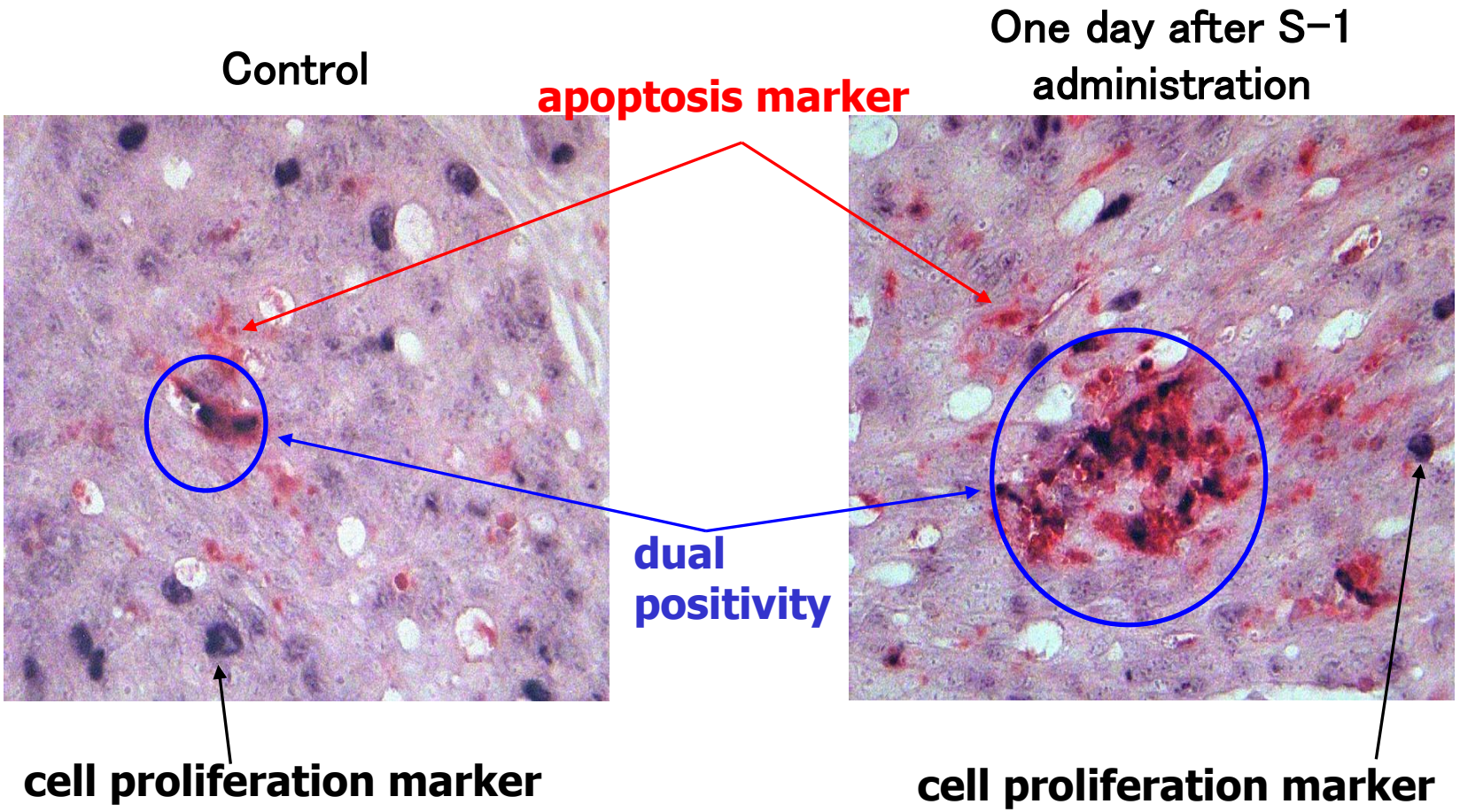
(Heating pretreatment in 1 mM EDTA solution, pH 8)

①Cell proliferation marker:

Ki-67(NBT/BCIP color→BLACK)

②Apoptosis markers:

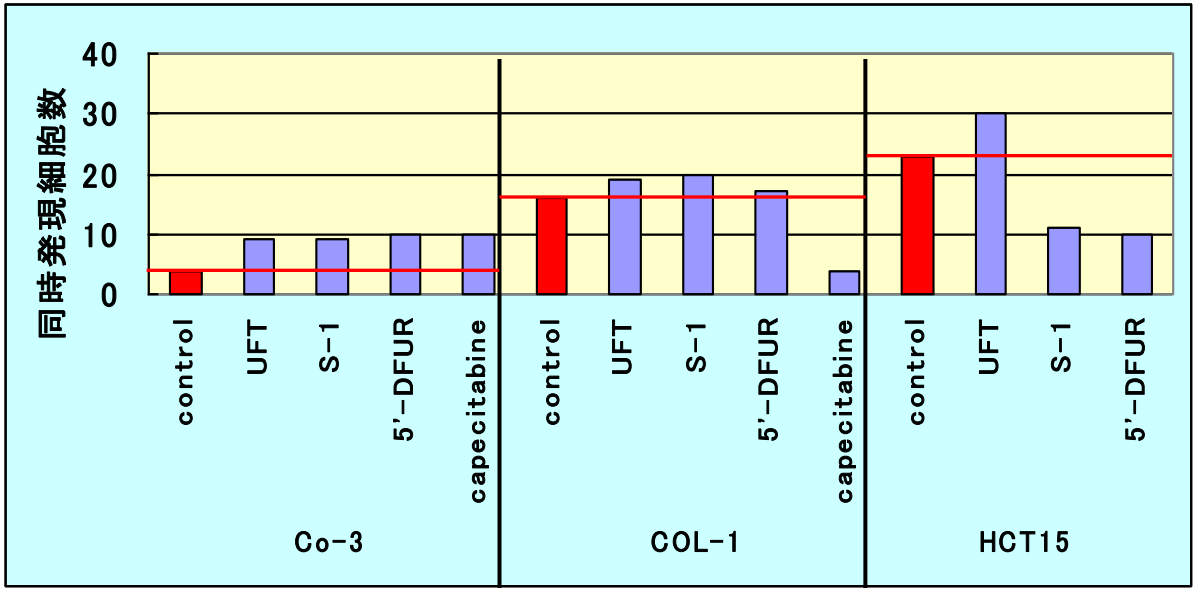
cleaved caspase 3 (Fuchsin color→RED)

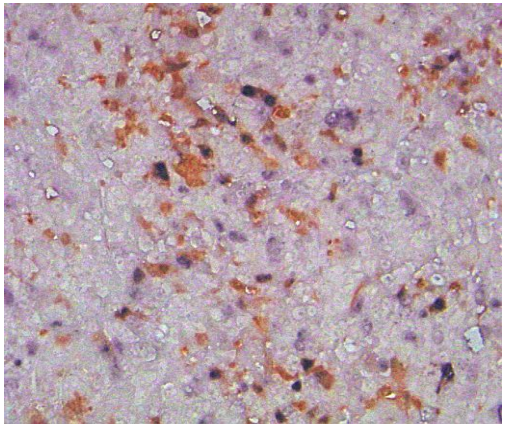


cell proliferation marker：Ki-67（NBT/BCIP coloration: black）：nuclear positivity
apoptosis marker： cleaved caspase 3 （Fuchsin: red）： cytoplasmic positivity
cleaved cytokeratin 18 （Fuchsin: red）： cytoplasmic positivity

Ki-67/cleaved caspase 3 dual positive cells in colon cancer xenograft (Co-3)

The number of proliferation/apoptosis dual positive cells in colon cancer xenografts

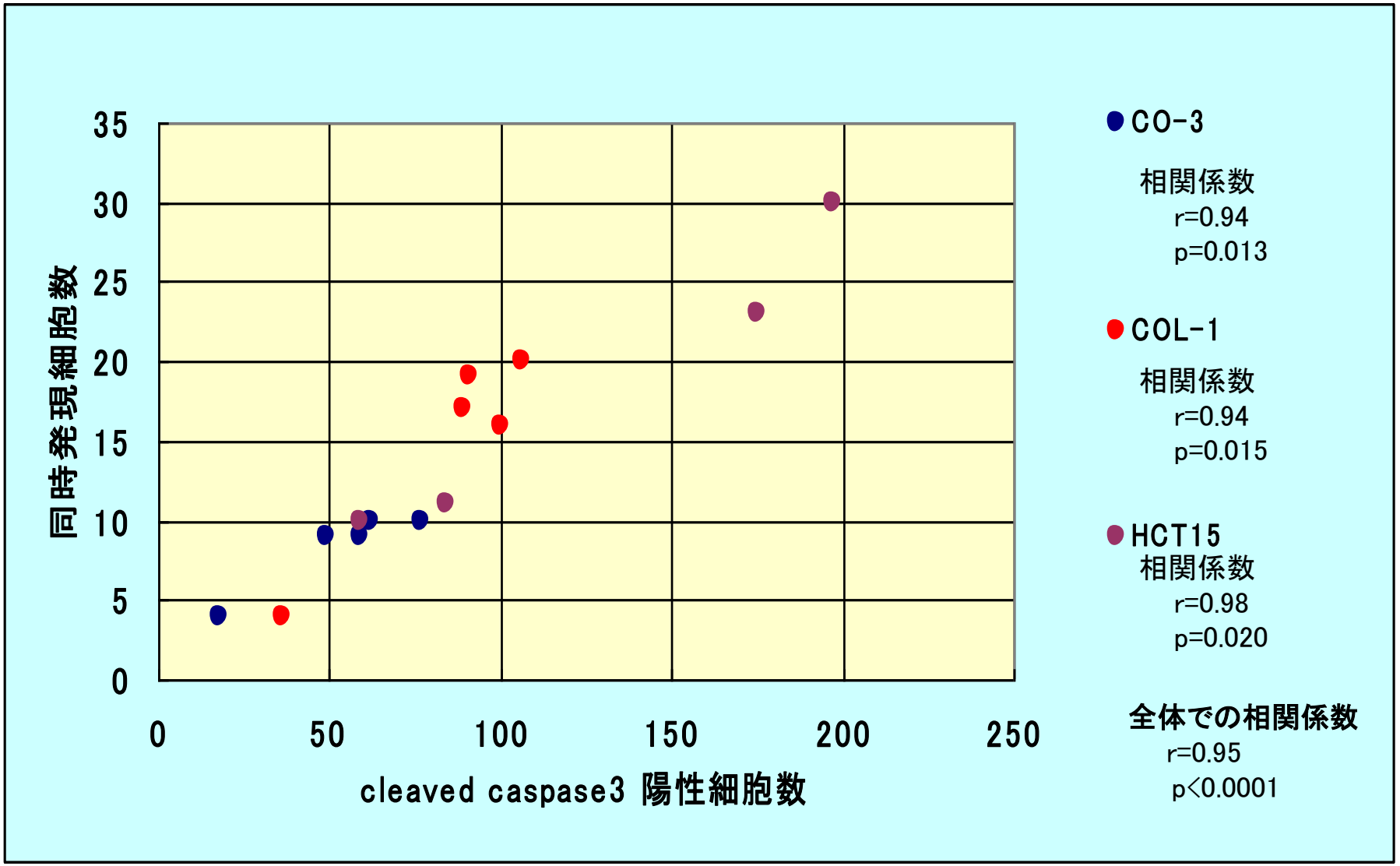




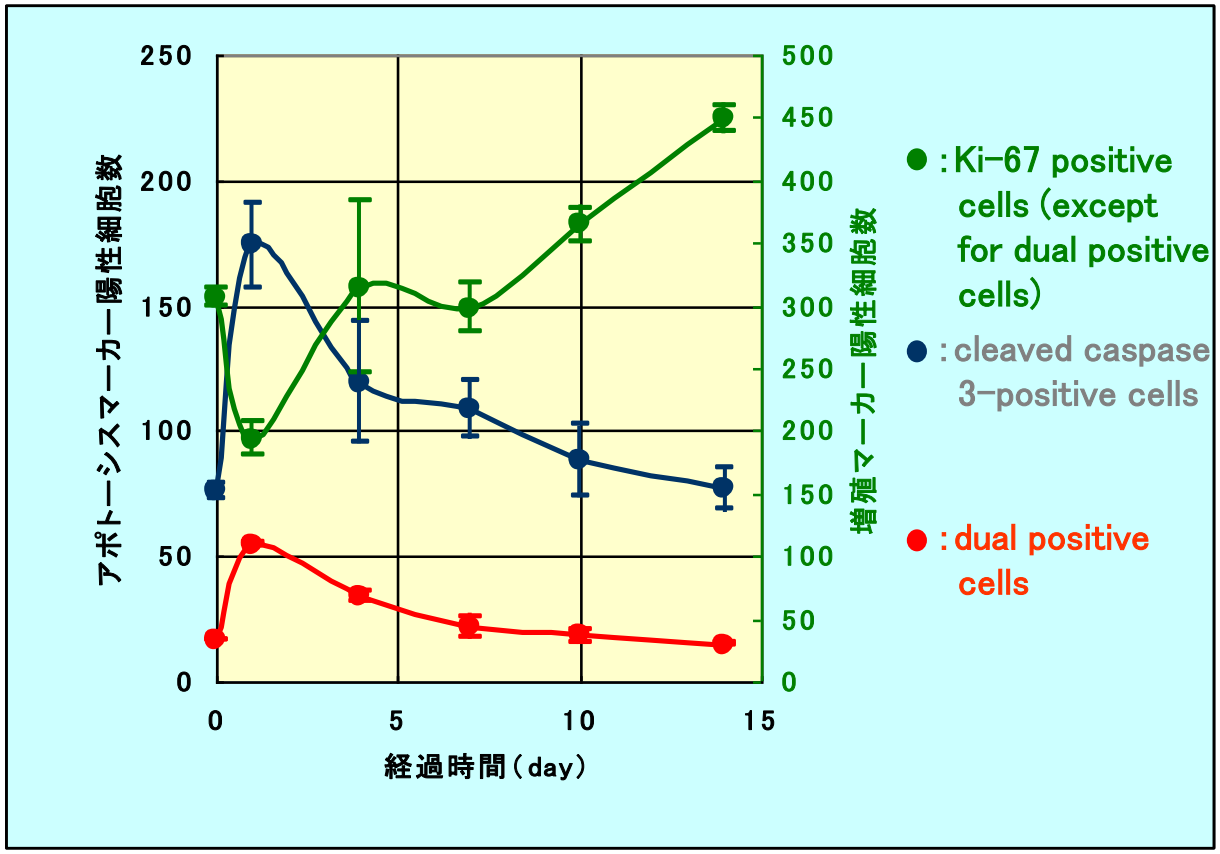
HCT15: anti-cancer drug NOT administered

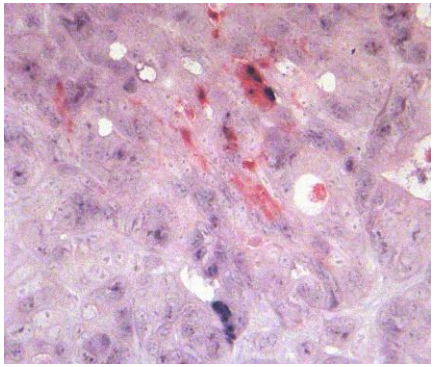
- Proliferation/apoptosis dual positive cells were NOT significantly increased after anti-cancer drug administration.
- The dual positive cells shared 16.4±6.6% of cleaved caspase 3-postive cells.
- The dual positive cells and the cleaved caspase 3-positive cells were correlated.

Correlation between cleaved caspase 3-positive cells and Ki-67/cleaved caspase 3 dual positive cells

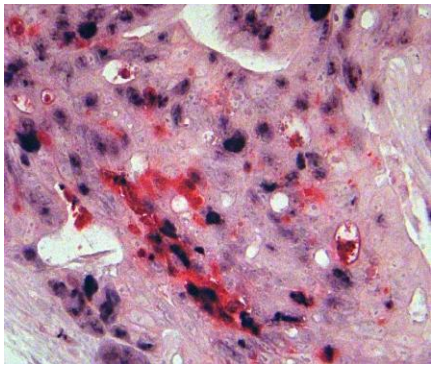


Time course of change of the number of proliferation/apoptosis dual positive cells in colon cancer xenograft COL-1 after 5-FU administration





Cntrol tumor



1 day after administer

・The numbers of dual positive cells and cleaved caspase 3-positive cells are closely correlated (correlation efficiency: $r=0.98$, $p<0.0001$)

・The number of dual positive cells and cleaved caspase 3-positive cells showed peaks at one day after 5-FU administration., and both numbers decreased thereafter. In contrast, Ki-67-positive proliferative cells decreased just after 5-FU administration, and increased thereafter. Cell growth and apoptosis were reversely correlated.

Summary

1) Cells simultaneously showing apoptotic and proliferation markers ("apoproliferative cells") can be demonstrated histochemically.

2) "Apoproliferative cells" appeared in correlation with cleaved caspase 3-positive apoptotic cells.

3) "Apoproliferative cells" in colon cancer tissue showed a peak number shortly (1-4 days) after 5-FU administration.

4) Proliferative activity was evidently suppressed when the "apoproliferative cells" showed a peak incidence.

Conclusions

1) 5-FU administration provokes apoptosis in mitotic cells. This phenomenon was visualized by double labeling of Ki-67 and cleaved caspase 3.

2) In colon cancer, the cleaved caspase 8-mediated death receptor pathway was the main route of apoptosis after 5-FU administration. In gastric cancers, some but not all showed cleaved caspase 9-mediated mitochondrial route of apoptosis.