Small-molecule activation of procaspase-3 to caspase-3 as a personalized anticancer strategy

Paul J. Hergenrother and Co-workers

Department of Chemistry
Department of Biochemistry
Univeristy of Illinois-Urbana

Nature Chemical Biology ***2006****, 2,* 543

Caspase: cysteine-aspartic-acid-protease

'The Executioner Proteins'

Apoptosis Signal Pathways: The Language of Cell Death

-All cells have apoptotic machinery, necessary for continued growth and elimination of faulty or malfunctioning cells.

-Cancer cells have a notable *resistance* to common apoptotic signals, allowing their continued existence and reproduction.

-Fundamentally, resistance is often a result of *improper regulation of critical proteins* and/or their genes in the apoptotic cascade.

-There are two types of apoptosis: *intrinsic* and *extrinsic*.

-Extrinsic: Cellular damage and/or death receptor activation/caspase-8
-Intrinsic: Signals that flow through mitochondria/caspase-9

-The *apoptotic signal cascade is complex* and not conserved in all cancers.

The Apoptotic Cascade: Intrinsic and Extrinsic Initiation and Convergence

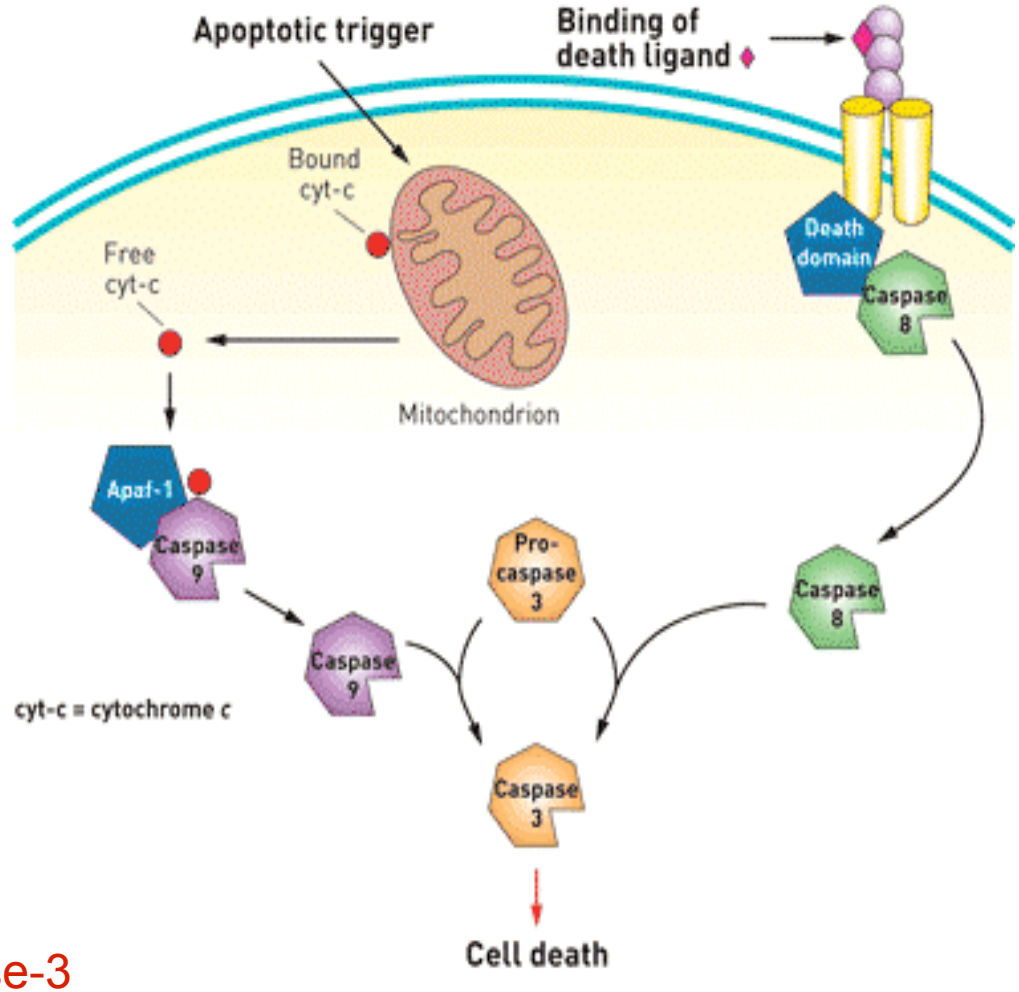
-This simplified scheme depicts *intrinsic* and *extrinsic* initiation.

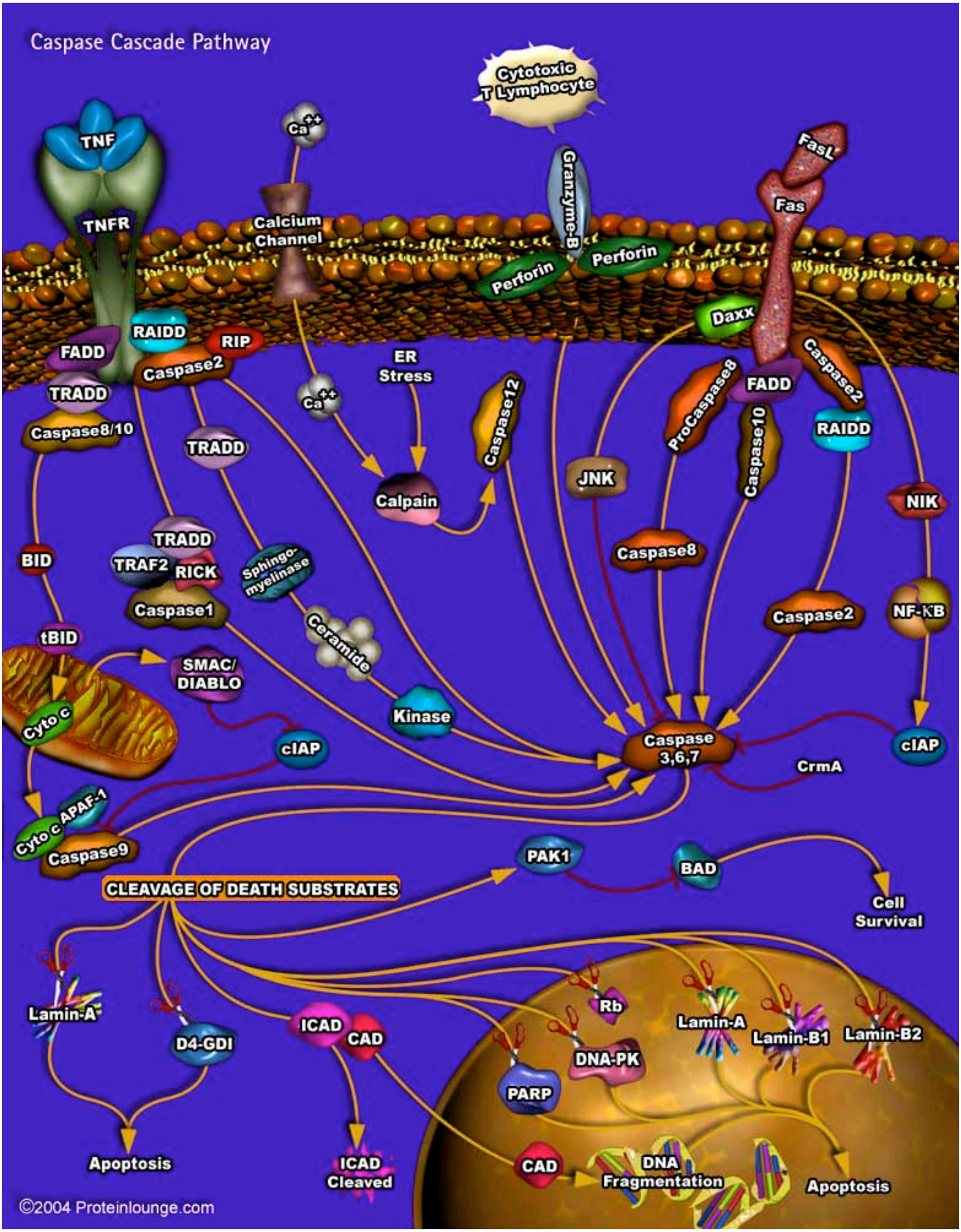
-Multiple caspases are involved in the pathways.

-Ultimately, most pathways process through caspase-3.

-Upstream signals in cancer can often be disrupted before processing all the way to execution.

-What does this mean? Caspase-3 is a target for small-molecule activation.





Apoptosis Signal Pathways: The Language of Cell Death

-Goal: Activate a proapoptotic signal far downstream.

-A therapeutic strategy targeting these proteins would be more successful since cancers have highly elevated levels of these proteins (1-6 fold).

Procaspase-3 $\xrightarrow{\text{Caspase-8 or -9}}$ ***Caspase-3***, *the active executioner*

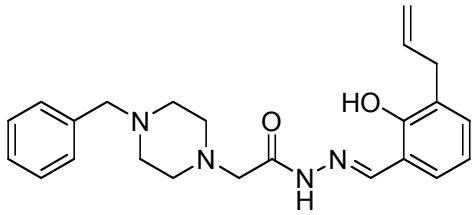
-Procaspase-3 has a Ile-Glu-Thr-Asp 'active site' blocked by Asp-Asp-Asp 'safety'

Concept: Screen for 'safety' cleavage, which releases the executioner.

-20,500 compounds screened against Asp-Glu-Val-Asp-p-nitroanilide.

-Observe at 405 nm for p-nitroaniline, indicating cleavage.

Activation of Procaspase-3 by PAC-1: Discovery

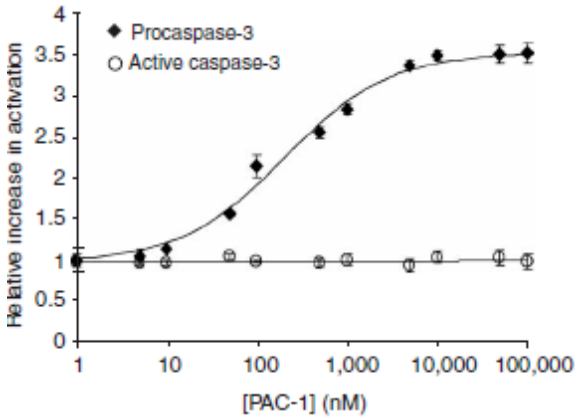


PAC-1

Cleavage of procaspase-3 to active caspase-3 by PAC-1.

-Of the 20,500 compounds, four showed activity and only one of those had a dose-dependant effect.

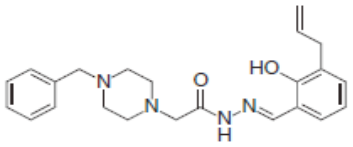
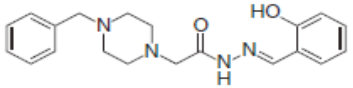
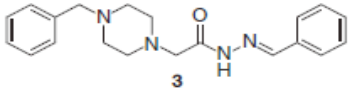
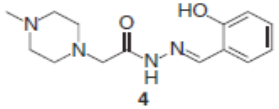
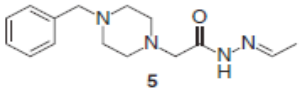
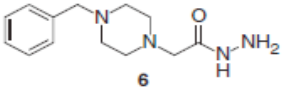
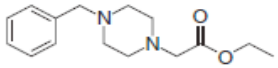
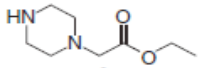
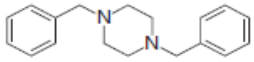
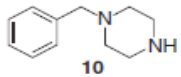
EC_{50} = 0.22 μM



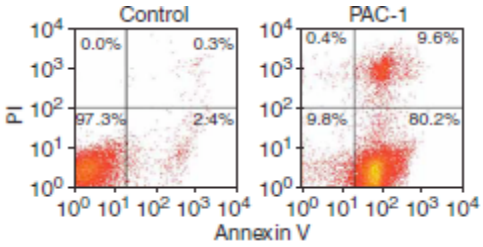
Relative activation of procaspase-3 by PAC-1

-Further testing in cancer cells was performed.

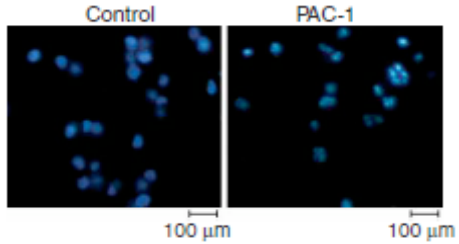
PAC-1: Activity in Cancer Cells

Compound	EC_{50} (μM) for procaspase-3 activation	IC_{50} (μM) for death induction in HL-60 cells
 PAC-1 (**1**)	0.22	0.92
 De-allyl PAC-1 (**2**)	0.43	1.74
 **3**	>50	>100
 **4**	>50	>100
 **5**	>50	>100
 **6**	>50	>100
 **7**	>50	>100
 **8**	>50	>100
 **9**	>50	>100
 **10**	>50	>100

PAC-1 shows many apoptotic hallmarks



-Cells lose the ability to distribute phospholipids in the membrane.



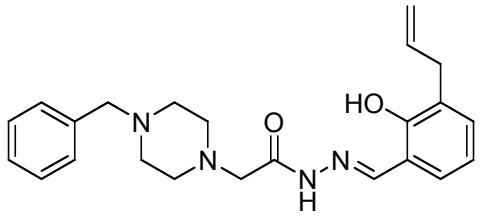
-Condensation of chromatin.

PAC-1 Activity in Other Cancer Cell Lines

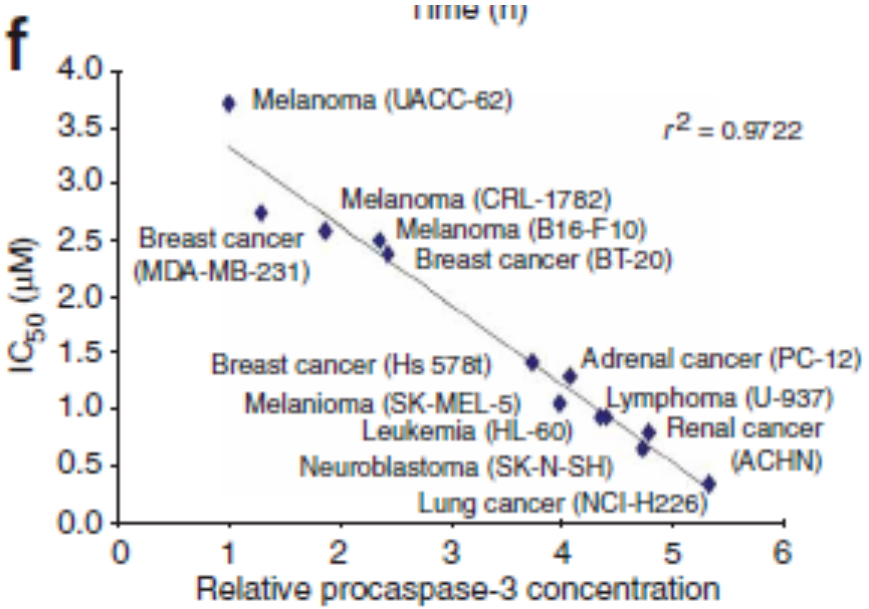
-PAC-1 was tested in cancer cell lines with varying amounts of procaspase-3.

-There is a strong correlation between IC_{50} and procaspase-3 concentration.

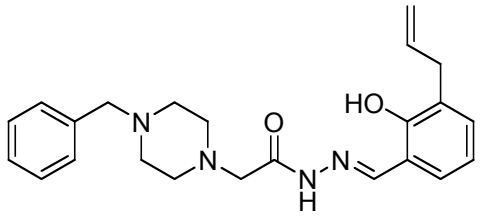
-Most potent in NCI-H226 (lung cancer) IC_{50} of 0.35 μM. This cell line has a procaspase-3 concentration of 5 times baseline levels.



PAC-1



PAC-1: Testing in Freshly Resected Colon Cancer Lines

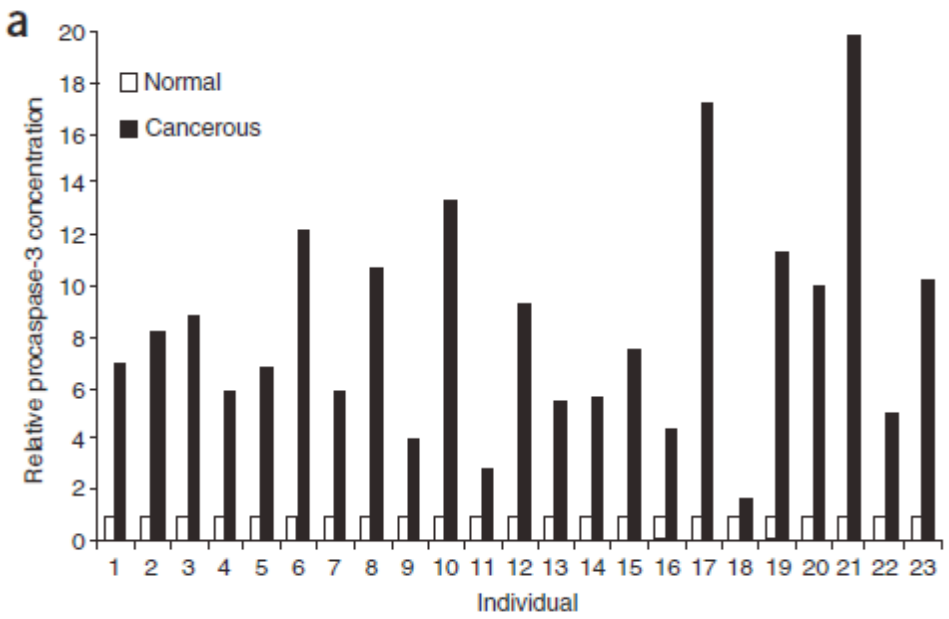


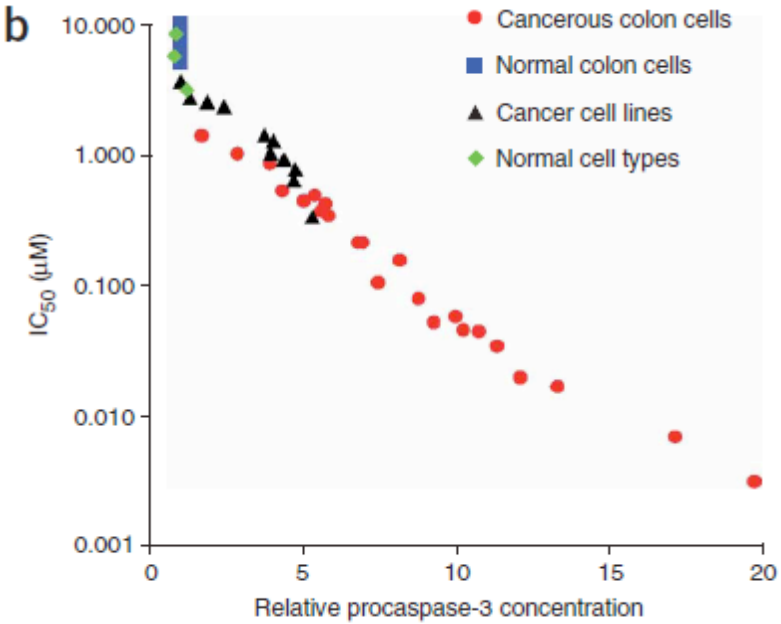
PAC-1

-In all cases, the cells had elevated levels of procaspase-3 (1.7-19.7 fold).

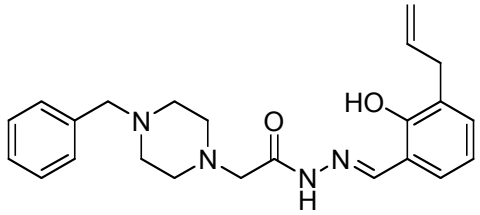
-IC_{50} values ranged from 0.003 to 1.41 μM.

-The cancer cells were 2000 times more sensitive than surrounding normal cells.





PAC-1: Testing in Freshly Resected Colon Cancer Lines and Nearby Normal Tissue



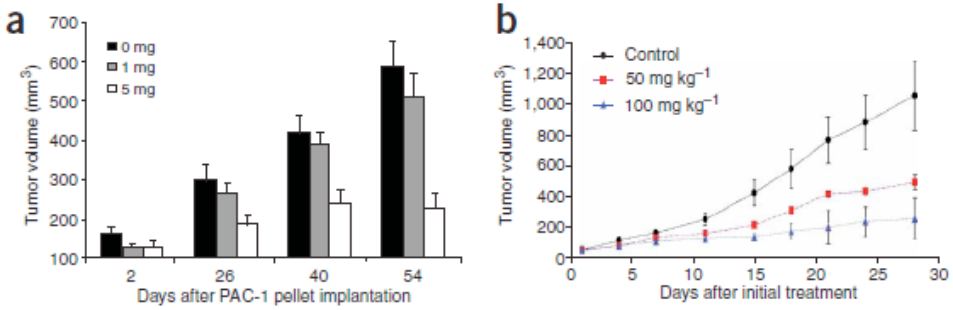
PAC-1

-Individual IC_{50} data from colon cancer/normal tissue screen. →

Table 1 IC_{50} values of PAC-1 versus cells isolated from primary cancerous and noncancerous colon tissue

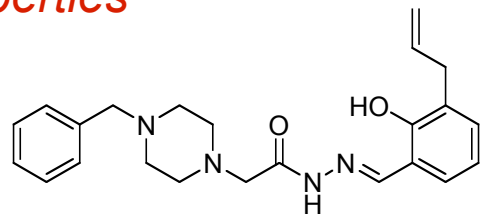
	PAC-1 IC_{50} (μM)	
Individual	Normal	Cancerous
1	6.78	0.212
2	9.79	0.154
3	6.61	0.080
4	9.50	0.340
5	6.88	0.216
6	6.28	0.020
7	7.34	0.422
8	5.67	0.045
9	6.54	0.844
10	9.98	0.017
11	5.94	1.030
12	5.63	0.052
13	5.50	0.499
14	7.58	0.366
15	5.96	0.106
16	5.02	0.527
17	5.17	0.007
18	6.39	1.410
19	5.41	0.034
20	6.84	0.058
21	6.25	0.003
22	5.73	0.439
23	5.28	0.046

-Direct implantation of PAC-1 via pellet in mouse tumor studies shows decrease in tumor volume over time (slow delivery).



Apoptosis Signal Pathways: Conclusions

-A potential approach to selective cancer therapy has been demonstrated by using the natural properties of cancer cells against themselves.



PAC-1

Caspase: cysteine-aspartic-acid-protease

'The Executioner Proteins'

EC_{50} = 0.22 μM