

## Controversy

## Resistance to apoptosis should not be taken as a hallmark of cancer

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## Abstract

In the research community, resistance to apoptosis is often considered a hallmark of cancer. However, pathologists who diagnose cancer via microscope often see the opposite. Indeed, increased apoptosis and mitosis are usually observed simultaneously in cancerous lesions. Studies have shown that increased apoptosis is associated with cancer aggressiveness and poor clinical outcome. Furthermore, overexpression of Bcl-2, an antiapoptotic protein, is linked with better survival of cancer patients. Conversely, Bax, CD95, Caspase-3, and other apoptosis-inducing proteins have been found to promote carcinogenesis. This notion of the role of apoptosis in cancer is not new; cancer cells were found to be short-lived 88 years ago. Given these observations, resistance to apoptosis should not be considered a hallmark of cancer.

**Key words** Cancer, apoptosis, Bcl-2, hallmark

Cell death is a mystery to cancer just as death is to humans. The world population grew slowly over thousands of years but dramatically increased in the last century, primarily because food supplies were sufficient, deadly diseases were effectively controlled, and the world was generally at peace. These factors prolonged the average lifespan, and as a result, the world population thrived.

Along these lines, scientists reasonably conjectured that the prolonged lifespan of cells might contribute to carcinogenesis. The idea first emerged in Kerr's original paper in which he proposed the concept of apoptosis<sup>[1]</sup>. The finding of Bcl-2 as an apoptosis suppressor almost surely bound cancer with the notion of being anti-apoptotic. Anti-apoptotic genes have since been considered a new category of oncogenes<sup>[2]</sup>. Gradually, evasion of apoptosis evolved to become a hallmark of cancer and became deeply rooted in the minds of cancer scientists<sup>[3,4]</sup>. Conversely, apoptosis has been considered a barrier to carcinogenesis<sup>[5]</sup>.

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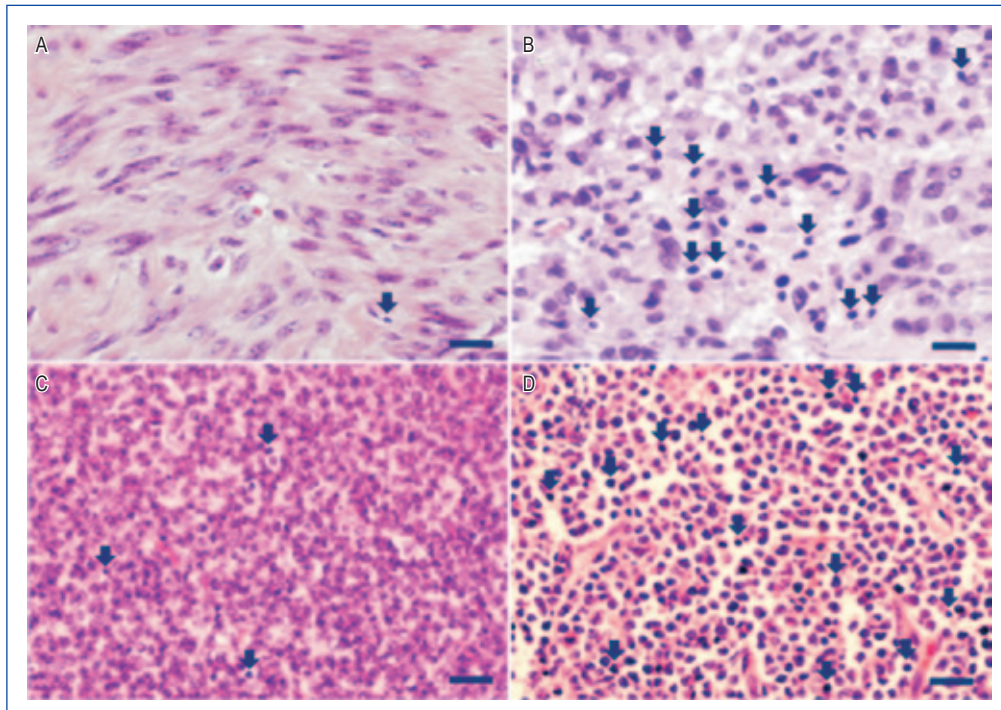
## Occurrence of Apoptosis in Solid Tumors

Cell death in cancer, which includes necrosis and apoptosis, is a far more complex issue than evasion of apoptosis. Cancer cells do not live longer than normal cells; rather, they have a shorter lifespan. Indeed, Dr. Alexis Carrel from Rockefeller Medical Institute stated in 1925, "malignant cells are sick cells which live shorter"<sup>[6]</sup>. This was confirmed again in 1937 by Fischer<sup>[7]</sup>.

What do pathologists see everyday under the microscope in cancer—more or less apoptosis? The answer is more apoptosis (**Figure 1**). Furthermore, the occurrence of apoptosis increases with the histologic grade of cancer, i.e., the higher the grade of the malignancy, the more apoptosis. Detailed studies were done by Dr. Lipponen's group from Finland<sup>[8-10]</sup> and by Krajewski *et al.*<sup>[11]</sup> from USA. They found apoptosis was positively related to histologic grade, cell proliferation indices, and poor patient survival in breast, colon, bladder, and other cancers<sup>[8-11]</sup>. A Japanese group also observed similar results in breast cancer<sup>[12]</sup>. Unfortunately, these important findings were not paid much attention because cancer researchers believed cancer cells to be apoptosis-resistant.

## Inconsistent Findings on the Clinical Roles of Bcl-2

Based on the observations noted above, it seems improper to consider cancer resistant to apoptosis. Nevertheless, opinions vary, both in publication<sup>[13]</sup> and in private. People who favor this as



**Figure 1. Apoptosis in benign and malignant tumors.** Routine paraffin sections were stained with hematoxylin and eosin. Apoptosis is characterized by condensed or broken nuclei (arrows). A, apoptosis is difficult to see in leiomyoma. B, apoptosis is highly frequent in leiomyosarcoma. C, low-grade follicular B-cell lymphoma, a type of relatively benign lymphoma, is characterized by Bcl-2 translocation, in which apoptosis is rare. D, comparing with the follicular B-cell lymphoma of left side, Burkitt lymphoma has much more apoptosis. The natural course of Burkitt lymphoma is much shorter than low-grade follicular B-cell lymphoma. These results indicate that apoptosis is much more frequently seen in highly malignant tumors than in relatively benign tumors. *Bar* = 20  $\mu$ m.

a hallmark may argue that there is less apoptosis in Bcl-2–positive B-cell lymphoma. Although this is a good argument, Bcl-2–positive B-cell lymphoma has properties that set it apart from most malignant diseases. More specifically, this type of lymphoma progresses very slowly, with most patients living more than 10 years after diagnosis. It also does not require special treatment. In contrast, the same histologic type of lymphoma without Bcl-2 overexpression is generally high grade with high frequencies of apoptosis and must be treated.

Bcl-2 is also overexpressed in tumors other than lymphoma. Interestingly, Bcl-2 overexpression is not related to poor clinical outcomes but to better outcomes in breast, colon, and lung cancers<sup>[14-17]</sup>. In a recent study, Bcl-2 was reported to be an effective predictor of good prognosis comparable to estrogen receptor in breast cancer<sup>[15]</sup>. Thus, the anti-apoptotic properties of cancer cells seem to also confer patients the ability to survive. Furthermore, both cell culture and transgenic mice studies have shown that Bcl-2 inhibited cancer cell growth and retarded carcinogen-induced tumorigenesis<sup>[18,19]</sup>.

Admittedly, there are also cancers, such as prostate cancer and hematologic cancers, in which Bcl-2 expression is related to poor clinical outcomes<sup>[20,21]</sup>. In normal prostate tissue, Bcl-2 is expressed in the basal cells of glands. In prostate cancer, Bcl-2–positive cells are also classified as basal type, which is androgen receptor (AR)–negative<sup>[20]</sup>. Thus, Bcl-2–positive prostate cancer is a special

case, not quite comparable to Bcl-2–negative prostate cancer. Hematologic cancers with Bcl-2 overexpression are more resistant to chemotherapy, similar to the aforementioned Bcl-2–positive B-cell lymphoma<sup>[21]</sup>. The natural course of Bcl-2–positive follicular B-cell lymphoma is much slower than that of Bcl-2–negative disease, but Bcl-2–positive lymphoma cannot be cured like Bcl-2–negative lymphoma.

## Other Paradoxical Molecular Findings

### Cell death receptor CD95 promotes cancer growth

Bcl-2 is not alone in this antiapoptosis enigma of cancer. CD95/Fas/Apo-1 is a death receptor that elicits apoptotic signals and functions in many physiologic and pathologic processes. Upon CD95 knockdown, cancer cells failed to grow both *in vitro* and *in vivo*<sup>[22]</sup>. Conversely, overexpression of CD95 facilitated tumor growth and was found to be associated with extremely poor outcomes of patients with melanoma or renal cancer<sup>[22-24]</sup>.

### Cell death executioner protein Caspase-3 is essential for tumor growth

Caspase-3 is the final executioner enzyme that, when activated,

destroys structural proteins of a cell. A Chinese group showed that when *caspase-3* was knocked down, tumors failed to grow<sup>[25]</sup>.

### Apoptosis-promoting protein Bax facilitates carcinogenesis

Bax, the Bcl-2-associated X protein, promotes apoptosis by releasing cytochrome *c* and other proapoptotic factors from mitochondria to cytoplasm. In the first version of their well known "Hallmarks of Cancer" report, Hanahan and Weinberg cited the anticancer effects of Bax as evidence of antiapoptotic feature of cancer<sup>[3]</sup>. However, this reference was removed in the second version of the paper<sup>[4]</sup>. Instead of a tumor suppressor<sup>[26]</sup>, Bax was later found to facilitate carcinogenesis<sup>[27]</sup>.

## Conclusions

The principle of scientific research is seeking truth from facts. By and large, neither evasion nor resistance to apoptosis is a feature of

cancer. Some may argue that proliferation still outweighs cell death in cancer. Evidence has long supported this claim, but this does not justify evasion of apoptosis as a hallmark of cancer. The key lesson to be learned from this cancer/antiapoptosis issue is that extreme reductionism can be misleading. We cannot say conclusively that cancers are antiapoptotic solely based on Bcl-2 overexpression; neither can we conclude that cancer cells have a short cell cycle time because Cyclin D1 is overexpressed and *TP53* is mutated. In most cases, cancer cells have a long cell cycle<sup>[28]</sup>. The molecular changes may rather be adaptive alterations than be the driving forces of malignant progression<sup>[29]</sup>. On the other hand, the longevity of life and fecundity are two repulsive genetic traits, i.e., the shorter the life span, the higher the fecundity. As Ames stated in his triage theory that "nature always favors short-term survival over long-time survival"<sup>[30]</sup>, the more logical deduction is that increased cell death drives cancer cell proliferation and, thus, is the impetus of cancer growth<sup>[31]</sup>.

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## References

- [1] Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer*, 1972,26:239–257.
- [2] Korsmeyer SJ. Bcl-2 initiates a new category of oncogenes: regulators of cell death. *Blood*, 1992,80:879–886.
- [3] Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell*, 2000,100:57–70.
- [4] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*, 2011,144:646–674.
- [5] Green DR, Evan GI. A matter of life and death. *Cancer Cell*, 2002,1:19–30.
- [6] Carrel A. Mechanism of formation and growth of malignant tumors. *Ann Surg*, 1925,82:1–13.
- [7] Fischer A. The theory of the developmental physiology of malignant tumors. 1937,31:1–20.
- [8] Lipponen PK, Aaltomaa S. Apoptosis in bladder cancer as related to standard prognostic factors and prognosis. *J Pathol*, 1994,173:333–339.
- [9] Lipponen P, Aaltomaa S, Kosma VM, et al. Apoptosis in breast cancer as related to histopathological characteristics and prognosis. *Eur J Cancer*, 1994,30A:2068–2073.
- [10] Lipponen P. Apoptosis in breast cancer: relationship with other pathological parameters. *Endocr Relat Cancer*, 1999,6:13–16.
- [11] Krajewski S, Krajewska M, Turner BC, et al. Prognostic significance of apoptosis regulators in breast cancer. *Endocr Relat Cancer*, 1999,6:29–40.
- [12] Nishimura R, Nagao K, Miyayama H, et al. Apoptosis in breast cancer and its relationship to clinicopathological characteristics and prognosis. *J Surg Oncol*, 1999,71:226–234.
- [13] Enderling H, Hahnfeldt P. Cancer stem cells in solid tumors: is "evading apoptosis" a hallmark of cancer? *Prog Biophys Mol Biol*, 2011,106:391–399.
- [14] Callagy GM, Pharoah PD, Pinder SE, et al. Bcl-2 is a prognostic marker in breast cancer independently of the Nottingham Prognostic Index. *Clin Cancer Res*, 2006,12:2468–2475.
- [15] Callagy GM, Webber MJ, Pharoah PD, et al. Meta-analysis confirms BCL2 is an independent prognostic marker in breast cancer. *BMC Cancer*, 2008,8:153.
- [16] Sinicrope FA, Hart J, Michelassi F, et al. Prognostic value of bcl-2 oncoprotein expression in stage II colon carcinoma. *Clin Cancer Res*, 1995,1:1103–1110.
- [17] Tomita M, Matsuzaki Y, Edagawa M, et al. Prognostic significance of bcl-2 expression in resected pN2 non-small cell lung cancer. *Eur J Surg Oncol*, 2003,29:654–657.
- [18] Knowlton K, Mancini M, Creason S, et al. Bcl-2 slows *in vitro* breast cancer growth despite its antiapoptotic effect. *J Surg Res*, 1998,76:22–26.
- [19] Murphy KL, Kittrell FS, Gay JP, et al. Bcl-2 expression delays mammary tumor development in dimethylbenz(a)anthracene-treated transgenic mice. *Oncogene*, 1999,18:6597–6604.
- [20] Chaudhary KS, Abel PD, Lalani EN. Role of the Bcl-2 gene family in prostate cancer progression and its implications for therapeutic intervention. *Environ Health Perspect*, 1999,107 Suppl 1:49–57.
- [21] Droin N, Guery L, Benikhlef N, et al. Targeting apoptosis proteins in hematological malignancies. *Cancer Lett*, 2013,332:325–334.
- [22] Chen L, Park SM, Tumanov AV, et al. CD95 promotes tumour growth. *Nature*, 2010,465:492–496.
- [23] Macher-Goeppinger S, Bermejo JL, Wagener N, et al. Expression and prognostic relevance of the death receptor CD95 (Fas/APO1) in renal cell carcinomas. *Cancer Lett*, 2011,301:203–211.
- [24] Ugurel S, Rappal G, Tilgen W, et al. Increased soluble CD95 (sFas/CD95) serum level correlates with poor prognosis in melanoma patients. *Clin Cancer Res*, 2001,7:1282–1286.
- [25] Huang Q, Li F, Liu X, et al. Caspase 3-mediated stimulation of

- tumor cell repopulation during cancer radiotherapy. *Nat Med*, 2011,17:860–866.
- [26] Yin C, Knudson CM, Korsmeyer SJ, et al. Bax suppresses tumorigenesis and stimulates apoptosis *in vivo*. *Nature*, 1997,385:637–640.
- [27] Knudson CM, Johnson GM, Lin Y, et al. Bax accelerates tumorigenesis in p53-deficient mice. *Cancer Res*, 2001, 61: 659–665.
- [28] Baserga R. The relationship of the cell cycle to tumor growth and control of cell division: a review. *Cancer Res*, 1965,25:581–595.
- [29] Wang RA, Yan QG. Molecular adaptation of cancer cells. In: Popescu LM, Hargens AR, Singal PK, eds. *Adaptation biology and medicine*, Vol. 7. New Delhi: Narosa Publishing House, 2013:131–138.
- [30] Ames BN. Prevention of mutation, cancer, and other age-associated diseases by optimizing micronutrient intake. *J Nucleic Acids*, 2010,2010:1–11.
- [31] Wang RA, Li QL, Li ZS, et al. Apoptosis drives cancer cells proliferate and metastasize. *J Cell Mol Med*, 2013,17:205–211.