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Novel roles of apoptotic caspases in tumor repopulation, epigenetic reprogramming, carcinogenesis, and beyond

Ruya Zhao[1] · Rayan Kaakati[1] · Andrew K. Lee[1,2] · Xinjian Liu[3] · Fang Li[3] · Chuan-Yuan Li[1,2,3]

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Abstract
Apoptotic caspases have long been studied for their roles in programmed cell death and tumor suppression. With recent discoveries, however, it is becoming apparent these cell death executioners are involved in additional biological pathways beyond killing cells. In some cases, apoptotic cells secrete growth signals to stimulate proliferation of neighboring cells. This pathway functions to regenerate tissues in multiple organisms, but it also poses problems in tumor resistance to chemo- and radiotherapy. Additionally, it was found that activation of caspases does not irreversibly lead to cell death, contrary to the established paradigm. Sub-lethal activation of caspases is evident in cell differentiation and epigenetic reprogramming. Furthermore, evidence indicates spontaneous, unprovoked activation of caspases in many cancer cells, which plays pivotal roles in maintaining their tumorigenicity and metastasis. These unexpected findings challenge current cancer therapy approaches aimed at activation of the apoptotic pathway. At the same time, the newly discovered functions of caspases suggest new treatment approaches for cancer and other pathological conditions in the future.

Keywords Apoptotic caspases · Non-canonical roles · Epigenetic reprogramming · Carcinogenesis · Sub-lethal caspase activation · Cancer stem cells

1 Apoptotic caspases, the established paradigm

Apoptosis is a process for multicellular organisms to get rid of damaged or unwanted cells. Apoptotic cells die in an orderly fashion with features of pyknosis, karyorrhexis, and karyolysis; no inflammation follows, which is a main characteristic that sets it apart from necrosis. There is no disturbance of neighboring cells, as apoptotic bodies are taken up by macrophages.

Caspases, a family of proteases that are pivotal executioners of apoptosis, comprise of the initiators and the effectors [1]. The initiator caspases include caspase-2, -8, -9, and -10, and the effector caspases include caspase-3, -6, and -7. The apoptotic process in mammalian cells is mediated through either the intrinsic or the extrinsic pathway. Briefly, the intrinsic pathway is usually activated as a result of pro-apoptotic signals that activate several proteins inside the mitochondria to be released into the cytoplasm. A key mitochondrial protein released being cytochrome c, which binds to APAF1 that causes APAF1 to further bind to ATP/dATP, forming the apoptosome [2]. The apoptosis then recruits and activates caspase 9. Of note, additional proteins that were released from the mitochondria in addition to cytochrome c include apoptosis-inducing factor (AIF), endonuclease G, high temperature requirement protein A2, second mitochondria-derived activator of caspase, and direct inhibitor of apoptosis binding protein with low pH [3]. Activation of caspase-9 leads to caspase-3 activation, which is the executioner caspase that kills the host cell.

On the other hand, the extrinsic pathway is initiated by extracellular signals. Extracellular molecules, such as the well-known Fas Ligand binds to the Fas receptor on cellular surface [4]. Upon binding, cytosolic factors caspase-8 and FADD associate with the activated homotrimeric receptor to form the death-inducing signaling complex or DISC [5]. Subsequently, DISC cleaves caspase 8 and allows it to activate the effector caspase 3, which leads to dismantling of critical cellular infrastructure and eventual cell death.

✉ Chuan-Yuan Li
Chuan.li@duke.edu

[1] Duke University School of Medicine, Durham, NC, USA

[2] Department of Pharmacology and Cancer Biology, Duke University Medical Center, Box 3135, Med Ctr, Durham, NC 27710, USA

[3] Department of Dermatology, Duke University Medical Center, Box 3135, Med Ctr, Durham, NC 27710, USA

In addition to eliminating damaged cells, programmed cell death is essential in morphogenesis and organ sculpting during development. Studies have used model organisms, including *Caenorhabditis elegans* [6], Drosophila [7], zebrafish [8], and mouse [9], to demonstrate that this role of apoptosis is highly conserved in evolution [10]. During gametogenesis, apoptosis operates to eliminate defective cells and limit the number of oocytes that are permitted to grow [11–15]. During organogenesis, apoptosis is pivotal in orchestrating the formation of the organs [16]. This programmed cell death also plays essential roles in development in humans, in regard to sex differentiation [17, 18], hearing acquisition [19], limb development [20], and immune system maturation [21].

Given the crucial roles of apoptosis, its disruption may be deeply problematic. Indeed, evading apoptosis is deemed a hallmark of cancer [22], and caspases are thought to be tumor-suppressive because of their roles for facilitating apoptosis. This makes sense intuitively, as cancer forms most often when p53 or BAX (both pro-apoptotic genes) are mutated [23]. In support of this view, the disruption of the apoptotic pathway has been implicated in many diseases, including neurodegenerative diseases [24–26], autoimmune diseases [27–29], and many types of cancer [30–33].

Based on the assumption that activating or enhancing the apoptotic pathway is beneficial for cancer treatment, much work has gone into developing therapeutic approaches to activate or re-enable the apoptosis pathway within cancer cells. One well-studied target is p53; the high percentage of mutated p53 in cancer and subsequent loss of its ability to induce apoptosis has led to much effort in finding a way to restore its normal function. Multiple approaches have been tested, including drug therapy, gene therapy, and p53-based immunotherapy, but as of yet, no clinical trial has yielded a noteworthy drug, and the FDA has yet to approve any p53-based therapeutic agent [34]. Another seemingly promising target is the IAP, with the reasoning that inhibition of the inhibitors of apoptosis may lead to greater induction of apoptosis *via* stressors. But despite promising results in xenografts and pre-clinical trials, no therapeutic agents targeting IAPs have yet progressed to the clinic either [35].

However, one avenue of investigation into activating the apoptosis pathway has led to a viable and approved therapeutic. BCL-2 is a protein that plays a critical role in the regulation of apoptosis as it prevents apoptosis by inhibiting pro-apoptotic molecules such as Bax and Bak [36]. Based on an understanding of the apoptotic mechanism and players, many pharmaceutical companies are looking to inhibit anti-apoptotic BCL-2 members and to use BH3 mimetics. Venetoclax, a small molecule that blocks Bcl-2 function, has been granted Breakthrough Therapy Designation by the FDA and approved for the treatment of chronic lymphocytic leukemia in patients with the 17p genetic mutation [37]. Letai et al. discussed the need to tailor the use of BH3 mimetics to specific cancers and genotypes. The authors developed "BH3 profiling" that allows for the determination of the cancer cell's dependence on specific anti-apoptotic proteins and predict sensitivity to BCL-2 antagonists [38, 39].

2 Novel roles of apoptotic caspases: promoting neighboring cellular proliferation and tissue regeneration

Despite the well-entrenched views on apoptotic caspases above, a series of recent unanticipated findings have shifted the views of them as purely executioners of cell death. Indeed, more and more they are viewed as mediators of a diverse array of biological functions. In one study, Li et al. proposed the "Phoenix Rising" pathway, in which the dying cells promote wound healing and tissue regeneration in a paracrine manner in mice [40]. Caspase-3 and -7 hold keys in triggering the release of the growth signal PGE_2 to promote stem or progenitor cell proliferation and tissue generation (Fig. 1). In the absence of either of these caspases, mice become deficient in wound healing and liver regeneration.

The role of apoptotic caspases in tissue regeneration is not unique in mice. It was previously discovered that damaged imaginal discs in *Drosophila* induce the proliferation of the neighboring viable cells *via* Dronc-dependent p53 pathway [41]. *Xenopus laevis* requires the activation of caspase-3 to regenerate its tail [42]. Apoptotic caspases were also found to control the tissue regeneration and remodeling process in planarian [43]. Decapitated *Hydra* was found to regenerate its head from growth signals induced by apoptotic cells *via* caspase-dependent activation of the Wnt3 pathway [44]. These discoveries in diverse organisms provide strong support for the counterintuitive hypothesis that tissue regeneration induced by apoptotic cell death may be an evolutionally conserved pathway in multi-cellular organisms.

3 Novel roles of apoptotic caspases: stimulating tumor cell repopulation

The pathway in which the apoptotic cells release growth factors to promote tissue regeneration is clearly beneficial in normal tissue regeneration and wound healing. However, the "Phoenix Rising" pathway was also found to be hijacked by cancer cells to repopulate tumors during radiotherapy [45]. Huang et al. found that apoptotic tumor cells generate potent growth signals to stimulate repopulation of cancer after radiotherapy, through the same caspase-activated $iPLA_2$-AA-PGE_2 axis that is observed in normal tissues (Fig. 1). Indeed, inhibiting caspase-3 activities caused significant tumor sensitivity to radiotherapy in xenograft tumors. Consistent with the animal studies, authors found that breast and head and neck

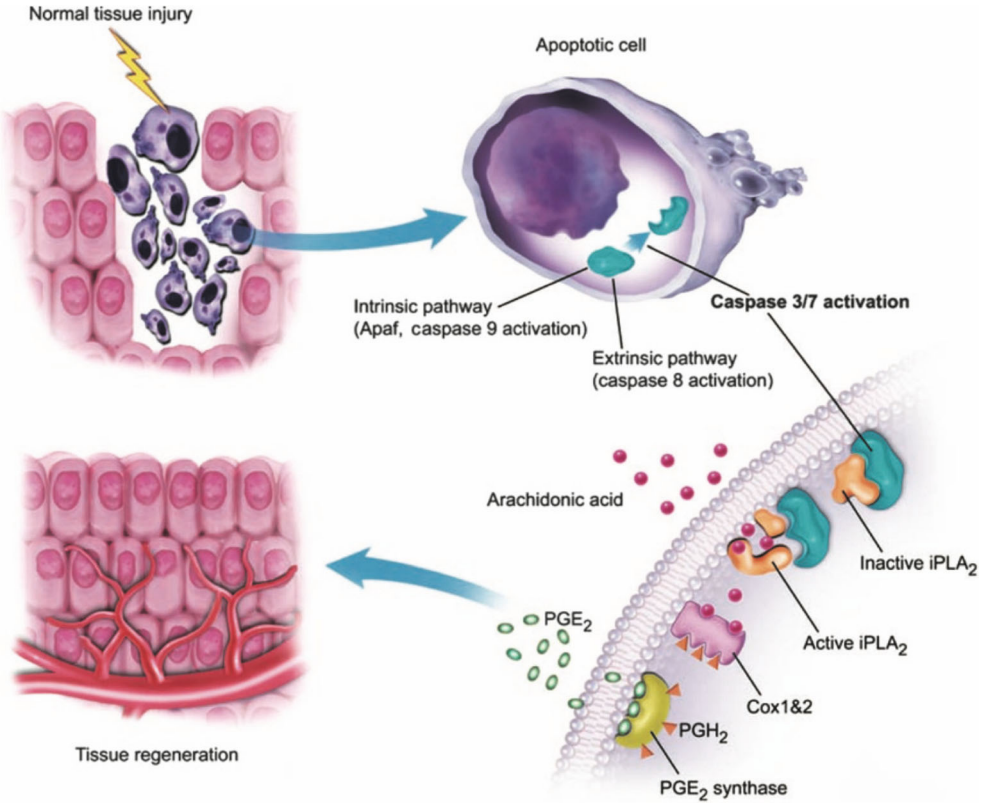

Fig. 1 A schematic representation of the "Phoenix Rising" pathway of cell death-induced tissue regeneration. In injured tissues, apoptotic cells activate caspase-3 and -7 through either the intrinsic or the extrinsic pathways. Activated caspase-3 and -7 subsequently cleave and activate $iPLA_2$, which generates arachidonic acid. Arachidonic acid is then converted into PGH_2 by cyclooxygenases 1 and 2 (Cox1&2). PGE_2 synthase converts PGH_2 into PGE_2, which stimulates stem cell proliferation and tissue regeneration. Adapted from ref. 40

cancer patients with higher levels of activated caspase-3 in tumor tissues demonstrated significantly increased rate of recurrence and deaths.

Caspase-3 has since been found to promote growth of surviving tumor cells in many types of cancer. Feng et al. uncovered that dying glioma cells promote post-irradiation angiogenesis in a caspase-3-dependent manner, and inhibition of caspase-3 ablated pro-angiogenic effects of dying glioma cells *in vivo* and *in vitro*. The authors identified the NF-κB-COX_2-PGE_2 axis as the key pathway mediating tumor response after irradiation [46]. In another study, Li et al. demonstrated that caspase-3 in dying tumor cells drove a pro-angiogenic response *via* VEGF-A and Akt signaling after irradiation [47]. Kurtova et al. revealed a mechanism in which bladder cancer stem cells actively contribute to chemoresistance *via* a proliferative response to repopulate killed tumor cells. This repopulation pathway was also found to be mediated by PGE_2 signaling [48]. Taken together, there is solid evidence from multiple groups to support the counterintuitive observation that apoptotic caspases promote neighboring tumor cell repopulation and confer therapeutic resistance in radiotherapy and chemotherapy.

Direct secretion of growth signals is not the only way that apoptotic cells use to communicate; they also secrete exosomes to maintain the viability of the neighboring cells. Exosomes are nano-sized lipid vesicles containing proteins, nucleic acids, tumor suppressor genes, micron RNAs, etc. These substances, particularly the nucleic acids, which are secreted both by tumor cells and the stromal cells can promote tumorigenesis and metastasis of cancers. Yu et al. found increased level of exosomal survivin, a protein that inhibits caspase activation, in breast cancer cells [49]. In a recent review paper, Lynch et al. discuss the specific role of apoptotic cell-derived EVs (Apo-EVs), which they define as heterogeneous vesicles ranging from 50 nm to several microns that are apoptosis dependent, which provide cells undergoing apoptosis with the capability of sending signals over significant distances. It has been shown that apoptotic bodies from tumor cells can induce p53 deficiency in fibroblasts, which promotes tumor growth, thus highlighting the possibility that cells that receive the Apo-EVs can transiently express the same genes from dying tumor cells and this may lead to sustained transformation [50, 51]. More research is needed, but it is clear that Apo-EVs are capable of producing important activating molecules that modulate the tumor's microenvironment.

4 Novel roles of apoptotic caspases: promoting metastasis

There is increasing evidence that apoptotic caspases are closely associated with cancer progression. A recent meta-analysis revealed that increased caspase-3 expression was statistically correlated with worse prognosis of breast cancer [52]. Zhou et

al. provided direct experimental evidence in support of a pro-metastatic role of caspases by demonstrating that colon cancer cells with *CASP3* knockout were markedly less invasive and more sensitive to radiotherapy *in vitro* and *in vivo*. More interestingly, cells deficient in caspase-3 were less prone to generate pulmonary metastasis when inoculated subcutaneously or intravenously. Authors also found significantly increased E-cadherin expression, reduced N-cadherin, Snail, Slug, and ZEB1 expression in caspase-3-deficient cancer cells, suggesting that the reduced EMT phenotype was implicated in the mechanism in which caspase-3 promoted metastasis [53]. Another recent study by Rudraptna et al. showed effector caspase activity drives cell invasion without initiating apoptosis in a Drosophila model. The study linked effector caspases to matrix metalloproteinase Mmp1 and Jnk pathway [54]. In the same vein of thinking, studies have also identified caspase-8 as a key regulator in integrin internationalization, cell motility, and metastasis [55]. Caspase-8 interacts with multiprotein complex to enhance cleavage of focal adhesion substrates and cell migration. Furthermore, caspase-8 knockdown disrupts metastasis in neuroblastoma *in vivo* [56]. Senft et al. reported that caspase-8 contributes to cell migration *via* interaction with p85, a subunit of phosphatidylinositol 3-kinase and an established cell migration component [57, 58]. In glioblastomas with poor response to radiochemotherapy, caspases were found to be constitutively active *in vivo* and *in vitro* in the absence of external stress or pro-apoptotic stimuli. Gydnia et al. reported that inhibition of caspase-3 and -8 decreased glioblastoma cell migration and invasion. This caspase-dependent motility was mediated by a constant cleavage of the motility-associated gelsolin protein [59]. In non-small cell lung cancers, elevated caspase-8 predicted early metastasis to the brain [60]. Such findings provide a rationale for elevated expression of apoptotic caspases in many malignant tumors in the absence of treatment. They also suggest inhibition of apoptotic caspases as a plausible approach to reduce cancer metastasis.

5 Activation of apoptotic caspase without cell death

Programmed cell death was previously believed to be irreversible after mitochondrial permeabilization and caspase activation [61–65]. Recent discoveries challenge the notion of the irreversibility of apoptosis and create a more complicated picture of our understanding of the conventional "apoptotic" caspases.

Tang et al. demonstrated the surprising results that even after cells underwent late-stage apoptosis, marked by mitochondrial permeabilization, caspase-3 activation, and DNA damage, the vast majority of cells actually recover when the inducers were washed away. This phenomenon was observed for many types of cells and inducers. The authors coined this unanticipated mechanism "Anastasia" [61]. Notably, some surviving cells acquired permanent genetic alterations and/or underwent oncogenic transformation. Independently, many other groups also investigated the roles of sub-lethal activation of effector caspases in various aspects including pluripotency maintenance, response to irradiation, genetic instability, and carcinogenesis. To more directly track the activation of caspase-3 *in vivo*, Ding et al. designed CasExpress, which drives fluorescent protein expression in cells that survive caspase-3 activation in Drosophila. Authors provided direct evidence of widespread sub-lethal caspase-3 activation in most tissues of every animal [65].

Sub-lethal activation of caspase activation is not without its consequences. Lovric et al. investigated the effects of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) on surviving glioma cells. TRAIL induces extrinsic apoptosis pathway by ligating death receptors that recruit FADD, caspase-8, and/or caspase-10 to form the "death-inducing signaling complex" [66]. Authors observed increased DNA damage and mutations in surviving tumor cells with activated caspase-8 after sub-lethal exposure to TRAIL or FasL [67]. Orth et al. demonstrated that prolonged mitotic arrest using antimitotic drugs partially actives the apoptotic pathway. This sub-lethal activation of caspases increased DNA damage by partly activating CAD, a downstream factor of caspase-3 [68].

6 Roles of non-lethal caspase activation in cellular differentiation

In *Drosophila*, it was found that caspases and cytochrome c aid the final stage of spermatid terminal differentiation, in which the bulk cytoplasm is removed. In human tissues, caspases are involved in the differentiation from human monocytes to macrophages [69]. Furthermore, caspase-8 plays a fundamental role in heart muscle differentiation [70], and caspase-14 was associated with terminal differentiation of keratinocytes [71, 72].

Fujita et al. reported that in order for embryonic stem cells (ESCs) to undergo germ layer-specific differentiation, caspase-3 is needed to induce cleavage of Nanog transcription factor. Stem cells lacking this *Casp3* gene showed marked defects in differentiation [73]. This finding is significant because Nanog has been shown in multiple studies to be important in maintaining pluripotency in stem cells. Silva et al. demonstrated that Nanog stimulated pluripotent gene activation from somatic cell genomes and increased Nanog is sufficient to reset neural stem cell epigenome to reset into a state of pluripotency [74]. Another study also found that Nanog expression resulted in increased ES-cell-like gene expression and DNA methylation patterns after inducing mouse fibroblast into iPS cells [75].

Dejosez et al. described another caspase-3-mediated ESC differentiation pathway. Their study showed that caspase-3 can cleave Ronin, a nuclear protein that possesses a zinc-finger DNA-binding motif (THAP domain), binds directly to HCF-1 (a key transcriptional regulator), and plays an essential role in embryogenesis and maintaining ESC pluripotency [76].

In addition to their role in ESC differentiation, caspases are also proven to be important in cell differentiation in other cell types. Kang et al. found that caspase-8 mediated differentiation in myelomonocytic lineage in bone-marrow cells [77]. Furthermore, a different group found that caspase-3 was required for RANKL-induced osteoclast differentiation. RANKL cleaves procaspase-3, and the activated protein is localized to the plasma membrane and cytosol. In procaspase-3 knockout cells or with caspase-3 inhibitors, primary osteoclasts failed to express TRAP or become multinucleated even with RANKL induction [78].

7 Roles of non-lethal activation of caspases in cellular de-differentiation

In addition to promoting cellular differentiation, caspases also facilitate the opposite process: de-differentiation. Li et al. reported that caspase-3 and -8 play critical roles in the induction of induced pluripotent stem (iPS) cells from human fibroblasts [79]. When Oct-4, a key iPSC transcription factor, is transduced in human fibroblasts, a portion of cells die in the reprogramming process. However, a significant number of cells with persistent caspase-3 activation survive and become iPSC. Surprisingly, inhibition of caspases-3 or -8 activation impeded iPSC induction, suggesting caspases-3 and -8 are necessary in the induction of pluripotent cells. The authors identified the tumor suppressor protein Rb as a key downstream target of Casp3/8 whose cleavage and inactivation plays key roles in epigenetic reprogramming. In addition to defining a key role for Casp3/8 in induction of iPSCs, the authors also observed consistently elevated expression of caspase-3 in iPSCs and H9 ESC, a well-known ESC line, consistent with the study of Fujita et al. [73].

At first glance, a role for effector caspases in both ESC differentiation and iPSC induction from human fibroblasts, which is basically a de-differentiation process, is puzzling. However, if one view caspases as an instrument of the cellular epigenetic machinery to go back and forth between differentiation and de-differentiation, it makes some sense. Obviously, many further studies are needed to clearly define the roles of caspases in regulating cellular differentiation and transcriptional reprogramming.

8 Role of non-lethal activation of caspases in maintenance of stemness and tumorigenicity of cancer cells

Cancer stem cells (CSCs) are important in tumorigenesis, metastasis, recurrence, and chemoresistance [80]. Cancer stem cells were shown to possess over-activated signaling pathways, including JAK/STAT, wnt/beta-catenin, Nanog, and Notch [81–85]. Even though they are often a very fraction of the overall cancer cell population, CSCs often play key roles in tumor growth and metastasis [86]. Moreover, CSCs were shown to be resistant to chemotherapy and radiotherapy [87–89]. However, the molecular mechanisms that sustain the stemness of cancer cells are now clearly defined.

In a recent study, Liu et al. demonstrated a surprising role of caspases in maintaining the tumorigenicity and stemness of breast cancer and glioma cells [90]. The authors found that many cancer cells have persistent effector caspase activation in the absence of any external stressors, much like those observed in iPSC and ESCs (Li et al., Fujita et al.). Similar to what is observed in ESCs, activated Casp3/7 did not kill the host cells. Instead, limited caspase activation leads to self-inflicted DNA double-strand breaks, which induces persistent ATM activation, resulting in Stat3 activation and in elevated CD133 expression in glioma CSCs [91].

The findings of Liu et al. directly challenges the established paradigm on the roles of apoptotic caspases in tumor growth and treatment. Furthermore, they also revealed the dark side of DNA damage response (DDR). They found that in many tumor cells, low level of cytochrome c leakage from the mitochondria leads to sub-lethal caspases activation, which causes DNA DSBs and activation ATM, a central player in the DDR. This occurs without exposure to any external stressors. When caspase-3, -6, and -7 were knocked out using CRISPR, the authors found significant reduction in DNA damage, reflected by gamma H2AX foci. Furthermore, caspases knockout breast tumor cells, EndoG/CAD (two apoptotic endonucleases downstream of Casp3/7 [92, 93]) knockout cells, and ATM knockout cells all showed significantly reduced tumor growth *in vivo*. Thus, authors established the functional relevance of caspases and spDSB on the growth and tumorigenic abilities of cancer cells (Fig. 2).

The identification of DNA damage/ATM activation/NF-kB/Stat3 activation/secretion of pro-tumor cytokines is reminiscent of the senescence-associated secretory phenotype (SASP) pathway, where the cell damage activates p53, triggering permanent growth arrest, which leads to the senescent state, and cells secrete signaling factors in the senescent state that leads to various pathological conditions including tumor promotion [94]. Similarly, our studies in tumor cells point to a pathway that the sub-lethal activation of caspase-3, -6, -7, and endonucleases (CAD and EndoG) leads to DNA double-strand breaking. This activates ATM and downstream factors

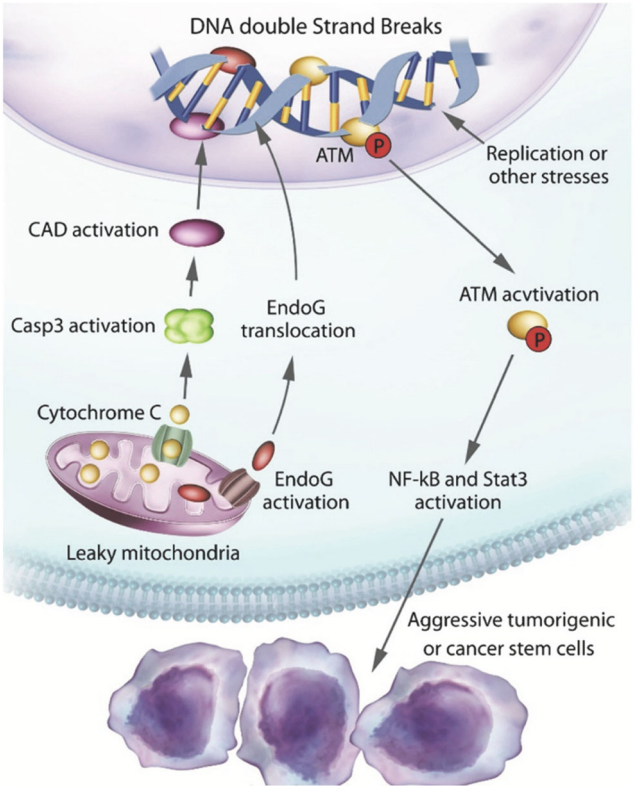

Fig. 2 An illustration of spontaneous DNA double-strand break induction and their roles in maintaining the stemness and tumorigenicity of cancer cells. Mitochondrial permeability changes in cancer cells allow spontaneous, sub-lethal activation of the apoptotic cascade that includes the cytoplasmic leakage of cytochrome c, caspase-3 activation, CAD activation, and EndoG nuclear translocation. The presence of spontaneous DNA double-strand breaks activates ATM, a key player in DNA damage repair pathway. Phosphorylated ATM activates NF-κB and Stat3, two factors well known to the maintenance of tumorigenicity and stemness of cancer cells. Adapted from ref. 88

(NF-kB and STAT3) and results in pro-inflammatory signaling factors secretion, which promote tumor growth (Fig. 2).

These unexpected roles of caspases in promoting cancer aggressiveness raise the questions on some of the current strategies of cancer therapeutics development. Thus, the singular approach of caspase activation in cancer treatment may need to be reassessed. In many instances, it might be beneficial to inhibit rather than activate effector caspases.

9 Role of sub-lethal caspase activation in carcinogenesis

Not only can caspases promote cancer aggression and metastasis, they may also be directly involved in promoting cancer development. Studies have found that pro-apoptotic pathways directly promote tumorigenesis in multiple types of malignancies. Biswas et al. demonstrated that loss of pro-apoptotic Bid surprisingly increases the latency of leukemogenic in Atm knockout mice. The authors suggest that a loss of Bid inhibits T cell tumorigenesis by increasing clearance of damaged cells [95]. Labi et al. also found that apoptosis actively drives tumor formation. The investigators demonstrated that mice defective in p53-induced apoptosis resist gamma-irradiation-induced lymphomagenesis, whereas repeated irradiation in wild-type animals leads to lymphoma formation by inducing expansion of hematopoietic stem cells [96]. Another study showed that the loss of Puma, a pro-apoptotic BH3-only protein of the Bcl-2 family, ablated tumorigenesis [97]. These studies strongly support that apoptosis promote cancer through its ability to generate compensatory proliferation of neighboring cancer cells or provide a vacant niche for cancer growth [98].

Mitochondrial outer membrane permeabilization (MOMP) was historically considered the point of no return in the apoptotic mechanism because the release of mitochondrial proteins including cytochrome c activates caspases [99] and leads to rapid cell death. However, Ichim et al. found that instead of killing the cells, a minority MOMP actually induces caspase-dependent DNA damage, promoting genomic instability and tumorigenesis [100]. Consistently, by use of a non-invasive caspase-3 reporter, Liu et al. demonstrated importance of caspase-3 activation in radiation-induced and chemical-induced malignant transformation of mammalian cells [101]. With low-dose radiation, a significant fraction of mammalian cells not only survive despite caspase-3 activation, but the surviving cells also demonstrated persistent DNA damage up to 3 months after exposure. More importantly, authors provided evidence that caspase-3 facilitates oncogenic transformation of human mammary epithelial cells. After exposure to irradiation, MCF10A cells were cultured and plated into soft agar, a well-established method to examine anchorage-independent growth in malignant transformation [102]. These irradiated cells readily formed soft agar colonies, and those with higher caspase-3 activation formed colonies at a significantly higher frequency. Furthermore, this ability to form soft agar colonies was significantly attenuated when caspase-3 was inhibited. In addition to irradiation-induced carcinogenesis, the authors also showed that caspase-3 facilitated two-stage chemically induced skin carcinogenesis *in vitro*. EndoG was found to be the downstream factor of caspase-3 role in mediating radiation-induced DNA damage and transformation (Fig. 3).

The facilitative role of caspase-3 in carcinogenesis was also confirmed in Myc-induced transformation of human mammary epithelial cells [103]. In that study, the authors demonstrated that an overexpression of Myc oncogene induces chromosome aberrations and gammaH2AX foci in non-transformed human mammary epithelial cells in a

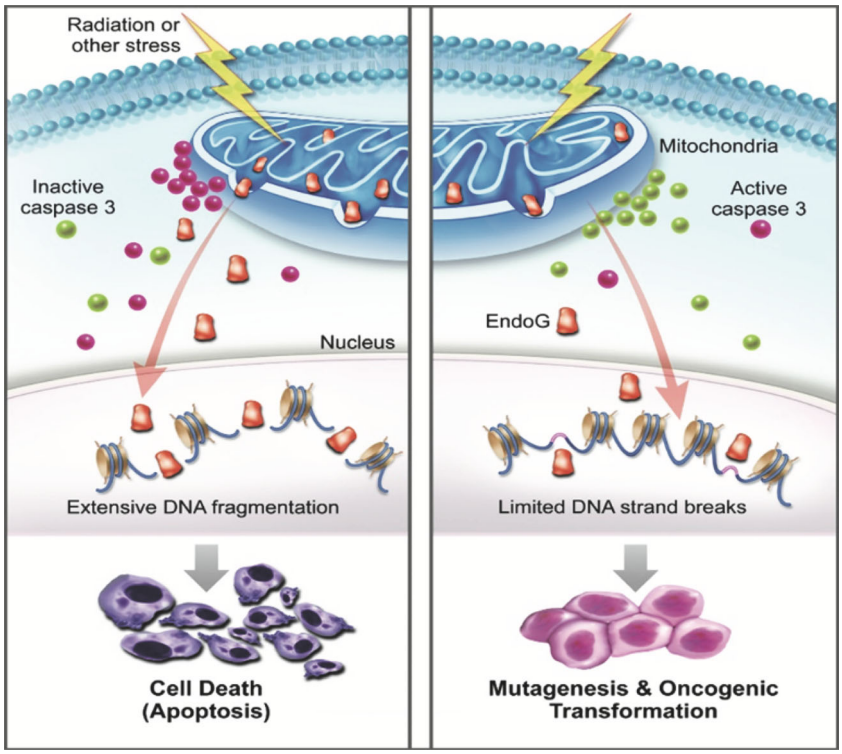

Fig. 3 A schematic diagram illustrating how abortive apoptosis facilitate stress-induced genetic instability and oncogenic transformation. Left panel shows the conventional scenario where mitochondrial permeability changes lead to activation of Casp3 and leakage of endonuclease G that kills the host cells. Right panel, on the other hand, shows partial leakage and survival of the cells with secondary genetic damage and oncogenic transformation

caspase-3-dependent manner. Furthermore, the authors show that Casp3 activation and activation of its downstream factor endoG is absolutely required for Myc-induced carcinogenesis. This finding thus resolves a long-standing dilemma in Myc-induced oncogenesis: Myc's role as both a powerful inducer of apoptosis and a powerful oncogenic factor.

10 Implications and future directions

Recent studies have significantly shifted the established paradigm based on the simplistic view caspases are strictly instruments of cell death. It is clear that they play unexpected roles in promoting, rather than suppressing carcinogenesis, epigenetic reprogramming, and genetic instability. One important clinical implication of these discoveries is that current anti-oncogenic therapies aimed at activating caspases to kill cancer cells are at best a flawed strategy. In fact, established cancer treatment such as radiotherapy and chemotherapy may select for cancer cells that could survive the treatments and become stronger by acquiring new mutations or become more stem cell-like based on sub-lethal caspase activation. Indeed, caspase inhibition may be a viable strategy to enhance current cancer therapy. In support of this view, new studies that look into caspase inhibition as an adjunct therapeutic approach have found promising results. Flanagan et al. demonstrated that selective inhibition of caspase-3 or its downstream effectors markedly reduced the expression of proliferation markers in colorectal tumor explant. Additionally, metastatic CRC patients with low level of

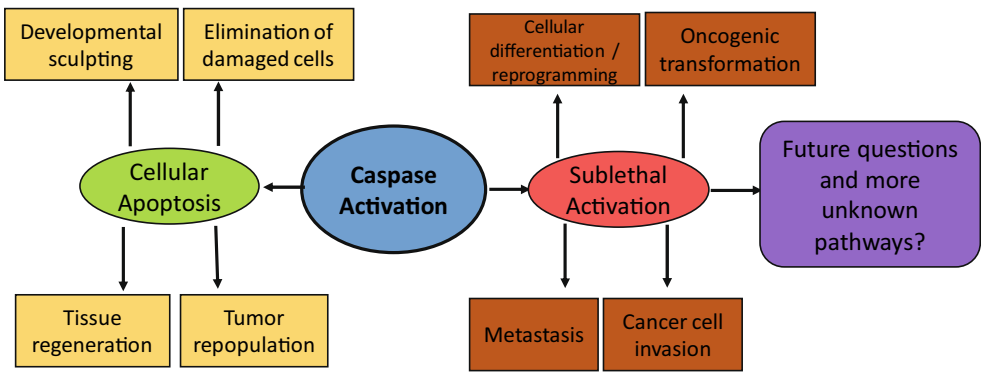

Fig. 4 A summary of the more established and recently identified non-canonical pathways related to apoptotic caspases

active caspase-3 had increased disease-free survival, especially in patients who received 5-FU chemotherapy [104]. All these findings point to a new direction of caspase inhibition as an adjunct anticancer option in advanced cancer.

Taken together, apoptotic caspases play a far more complicated role than simply being an instrument of cell death. More studies are clearly needed to achieve a better understanding of their non-lethal biological involvements, see (Fig. 4) for a summary. Only after achieving a better understanding can we begin to target them effectively to achieve therapeutic gains.

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