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Cell death-stimulated cell proliferation: A tissue regeneration mechanism usurped by tumors during radiotherapy

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Summary

Death of all the cancer cells in a tumor is the ultimate goal of cancer therapy. Therefore much of the current effort in cancer research is focused on activating cellular machinery that facilitates cell death such as factors involved in causing apoptosis. However, recently a number of studies point to some counter-intuitive roles for apoptotic caspases in radiation therapy as well as in tissue regeneration. It appears that a major function of apoptotic caspases is to facilitate tissue regeneration and tumor cell repopulation during cancer therapy. Because tumor cell repopulation has been shown to be important for local tumor relapse, understanding the molecular mechanisms behind tumor repopulation will be important to enhance cancer radiotherapy. In this review, we discuss our current knowledge of these potentially paradigm-changing phenomena and mechanisms in various organisms and their implications on the development of novel cancer therapeutics and strategies.

Introduction

Death is the fate for all cells in every living organism. While this is a universal truth, death can take many forms. A plethora of new terms, particularly in the last three decades, have emerged to describe the various modes of cell death [1]. Cell death plays a central role in cancer research because it is key in both cancer etiology and cancer therapy. During carcinogenesis, normal cells have to evade cell death signals to become tumorigenic. In cancer therapy, the ultimate goal is to achieve the death of all cancer cells in the patient's body. While the goal of killing cancer cells through radiation is very straightforward, there is increasing recognition that cell death and tumor repopulation, which is the opposite process of tumor cell loss caused by treatment, are closely intertwined. It appears that compensatory proliferation, a process initially identified in lower organisms during the regeneration of lost tissue [2, 3], is an evolutionarily conserved process that also functions in mammals. Moreover, tumors appear to have usurped this mechanism to their own advantage when dealing with cytotoxic cancer therapy. In this review, we attempt to summarize some recent advances in our understanding of the dynamic interactions between cell death and tumor repopulation; repopulation is a major reason for treatment failure during radiotherapy.

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We will start by reviewing some of the basic concepts of different modes of cell death. We will then review the phenomenon of compensatory proliferation during tissue regeneration in lower organisms. We will also take survey of recent literature on cell death-induced tissue regeneration and wound healing in mammalian organisms. Finally, we will examine results concerning the roles of apoptosis in tumor cell repopulation during radiotherapy and its potential implications for drug development and radiotherapy.

Cell death: the current paradigm

Broadly speaking there are three distinct modes of cell death: necrosis, apoptosis, and autophagy. Although all three pathways can achieve the simple endpoint, death of the cell, the molecular signaling cascades and consequences to the host can be highly varied. For example necrosis is often accompanied by an inflammatory response, which can promote tumor development, however the concomitant release of tumor antigens can also lead to an enhanced immune response through activation of immature dendritic cells and antigen-specific T cells to effectively combat tumor development [4]. Autophagy is even more complex as it has been shown to both promote and hinder tumor cell survival. On the other hand, apoptosis is considered a “clean” and rapid way of cell death where tumor cells quickly disintegrate and are phagocytized and degraded by macrophages in the tumor mass. It is thought that minimal inflammation is induced by apoptosis.

Necrosis is at first glance a chaotic, uncontrolled process leading to lysis of the cell. It is frequently considered as “accidental” cell death and most often occurs through external insults, either physical or chemical, that result in swelling of the cytoplasm, destruction of organelles and eventually bursting of the plasma membrane [5]. Consequently, intracellular contents are leaked out into the surrounding milieu resulting in an inflammatory response and tissue destruction that, in severe cases, can be fatal to the host [6]. Fortunately, not all cell death is so traumatic. The necessity of a more regulated mode of cell death to facilitate embryonic development and oversee normal cellular turnover while at the same time having minimal destruction to healthy tissues is achieved through programmed cell death [7].

Apoptosis, or programmed cell death, is well understood at the molecular level. It has been shown to be the main form of cell death in mammals. The term “apoptosis” first appeared in 1972 where it was used to describe a controlled process of cell death, distinct from that seen with necrosis, characterized by cell shrinkage, blebbing of the plasma membrane, chromatin condensation and DNA fragmentation [8]. Resultant membrane-bound smaller cellular fragments, known as apoptotic bodies, are then phagocytized by macrophages through recognition of phosphatidylserines that are externalized to the outer membrane of apoptotic bodies. Engulfment by macrophages occurs before their cellular contents can be leaked out into the surrounding environment. This efficient clean-up process effectively prevents an immune or inflammatory response, such as that seen with necrosis.

Apoptosis can occur through an extrinsic or intrinsic pathway, both of which carry out cellular substrate degradation through activation of caspases, a family of cysteine-proteases that cleave peptides adjacent to aspartic acid residues found within caspase-specific peptide sequences [9]. Activation of the extrinsic pathway occurs through engagement by specific ligands of cell surface death receptors, such as Fas, TRAIL, and TNF receptors [10]. The downstream signals vary depending on the receptor, but all of them culminate in the activation of effector caspases. For example, Fas receptor engagement facilitates recruitment of FADD and caspase-8 that make up the death-inducing signaling complex (DISC). This association cleaves caspase-8 yielding its active form which will go on to cleave and hence activate executioner caspases such as -3, -6, and -7.

In the intrinsic pathway, mitochondrial outer membrane permeabilization results in release of the mitochondrial protein, second mitochondria-derived activator of caspase/direct inhibitor of apoptosis-binding protein with low pI (Smac/DIABLO), into the cytosol where it competitively binds the Inhibitors of Apoptosis Proteins (IAPs) away from caspases allowing them to be activated [11]. Cytochrome C is also released from the mitochondria and, along with Apaf-1 and caspase-9, form the apoptosome. The initiator caspase-9 will, like caspase-8 in the extrinsic pathway, activate the executioner caspases such as caspase-3, -6, or -7, which themselves cleave a number of cellular substrates ultimately leading to death of the cell [11]. Often times, engagement of the extrinsic pathway will amplify the intrinsic pathway through caspase-8-dependent cleavage of the BH3-only protein, BID, which functions in the destabilization of the mitochondrial membrane [12].

Finally, the least well understood of these three mechanisms is autophagy. Autophagy by itself is not a cell death mechanism, rather it describes a process by which cells break down and recycle cellular components through fusing of phagocytic vacuoles with lysosomes. Please refer to the review by Koritzinski on its role in radiotherapy (this issue).

These three mechanisms are not without their overlap as crosstalk between these pathways has been well documented and can have dramatic consequences in the outcome of disease [13–15]. For example caspase activation can cause necrosis by promoting iron overload [16]. When caspases are inhibited and apoptosis blocked, some cells will switch to death by necrosis or autophagy. It was previously shown that treatment of murine fibrosarcoma cells L929 with the pan-caspase inhibitor, zVAD, to prevent apoptosis induction resulted in increased death by necrosis. However when cells were treated with zVAD plus rapamycin, which is known to induce autophagy, the cells survived. Furthermore, knockdown of selective autophagy genes resulted in enhanced zVAD-induced cell death through necrosis [17]. In other cases, autophagy induction after zVAD treatment led to increased cell death [18, 19]. The relationship between autophagy and apoptosis is particularly fluid as they have been reported to both amplify and hinder one another [20–23]. In the pursuit of effective cancer therapies, in which elimination (death) of the cancerous cells is the desired result, the mode of cell death induced and subsequent consequences to the host must be taken into consideration. Deregulated apoptosis is an established hallmark of cancer [24–27]. Often cancer cells develop resistance to apoptosis through down-regulation of death receptor expression or alterations in downstream signaling components. Therefore, exploiting other mechanisms of cell death, such as necrosis or autophagy, may be one way to get around this obstacle.

Compensatory proliferation, cell death induced tissue regeneration in lower organisms

Apoptosis is generally thought of as a means for multi-cellular organisms to get rid of damaged or unwanted cells. However, in recent years it was shown that apoptosis often plays key facilitative roles in tissue regeneration. This phenomenon, which was termed compensatory proliferation, was first observed in lower organisms, such as planaria, hydra, and drosophila. Planarians, which are simple worms, exhibit remarkable regenerative capabilities and can form complete individuals from the smallest of body parts [28]. In planarians, it is thought that up to 20% of all cells are committed to the regeneration of adult tissues [29]. Apoptosis-related genes, such as caspase-like gene 3 (DjCLg3), have been identified in apoptotic cells during planarian head and tail regeneration as well as during normal cellular turnover [30]. Hydra, like planarians, is one of the earliest models of regeneration; research with this model goes back hundreds of years. It also displays an exceptional capacity for regeneration. During Hydra head regeneration, there is a rapid onset of apoptosis and secretion of Wnt3 from the dying cells. Wnt3 is a member of the Wnt

family of signaling proteins whose activation has been implicated in oncogenesis in addition to regulating a number of developmental processes. This secreted Wnt3 activates the β -catenin pathway in surrounding cells, which leads to an increased rate of proliferation [31–33]. The importance of compensatory proliferation in head regeneration is demonstrated when blocking apoptosis through use of a caspase inhibitor prevents head regeneration from occurring. However, it can be restored if Wnt3 protein is exogenously added [32].

The role of compensatory cell proliferation in regeneration was further clarified in studies using drosophila. Imaginal discs are sacs of epithelial cells found in drosophila larva which will go on to become adult structures such as wings, eyes, and limbs. In drosophila wing imaginal discs, apoptosis is scarce under normal conditions, but is rapidly induced by radiation and heat shock. Of interest is the rapid repopulation of cells to produce normal adults, even with damage destroying up to 60% of the wing imaginal disc [34]. When such treatment is combined with an inhibitor of executioner caspases, the baculovirus P35 protein, to prevent the death of apoptotic cells, there is increased non-autonomous cell proliferation, invasion of compartments, and upregulation of the wingless (wg) and decapentaplegic (dpp) signaling genes [35–37]. The authors concluded that if apoptotic cells are not eliminated, they continuously emit Wg and Dpp signals, which results in developmental aberrations and postulated that a similar process of uncoupling apoptosis initiation and cell death may occur during tumor formation in mammalian cells [35].

In another drosophila study, Fan and Bergmann showed a second mechanism of apoptosis-induced compensatory proliferation occurring through activation of the hedgehog (Hh) signaling pathway by effector caspases, DrICE and Dcp-1, and that this is the preferred method in eye tissues [38]. Effector caspases in photoreceptor neurons were shown to stimulate Hh signaling causing cells that had previously exited the cell cycle to reenter it, thus highlighting how different caspases could trigger distinct forms of compensatory proliferation and exert nonapoptotic functions [38].

Moving to mammals, it was shown that mouse embryos exposed to the teratogen, mitomycin C, were reduced to 10% of their normal size, but were able to recover through a compensatory growth program, which resulted in normal sized embryos 48 hours later [39]. Taken together, these studies provide a rationale for investigation of the relationship between cell death and tissue regeneration in higher organisms.

Cell death stimulated skin wound healing and liver regeneration

As we have learned from observations made in lower organisms, increasing cell death in one cell population can inadvertently stimulate accelerated proliferation of surviving cells. Evolutionarily conserved aspects of this kind of signaling may very well be present in higher organisms for the purpose of wound healing and tissue regeneration. For example, just as the JNK pathway has been shown to play a role in drosophila imaginal disc compensatory proliferation, JNK activation may also play a role in promoting mammalian liver regeneration [40–42]. Maeda et al showed that in mouse hepatocytes where activation of the pro-survival NF- κ B pathway was inhibited, treatment with the liver carcinogen, diethylnitrosamine, lead to JNK activation and increased compensatory proliferation in the surviving hepatocytes [42]. In a follow up study by this same group, the importance of JNK activation to this process was confirmed by showing that mice lacking JNK had marked decreases in cyclin D, vascular endothelial growth factor, cell proliferation and tumor neovascularization [41].

In a recently published study we examined the effects of dying cells on tissue regeneration and wound healing in mice [43]. To simulate dying cells in wounded tissue we lethally irradiated mouse embryonic fibroblasts (MEF). Using a series of bioluminescence imaging

experiments to monitor the proliferation of firefly luciferase (Fluc)-labeled stem or progenitor cells, we found that co-culture with the irradiated MEFs led to a significant increase in proliferation of the Fluc-labeled epidermal keratinocyte progenitor cells. We were able to show that caspases-3 and -7 play key roles in this cell death-stimulated keratinocyte proliferation.

To determine if these caspases indeed played a role in wound healing, we created excision wounds through punch biopsies on mouse skin and compared the healing rates between wild-type and Casp3^{-/-} or Casp7^{-/-} knockout mice. While skin wounds of wild-type mice were completely healed by day 9, Casp3^{-/-} mice took 14 days to completely heal.

These caspases can also play a substantial role in liver regeneration. The human liver can regenerate up to 70% of itself without the formation of scar tissue [44] and we examined the importance of caspases in this process by utilizing a well-established partial hepatectomy model in mice [45]. After removing 2/3 of the total liver weight in wild-type and caspase knockout mice, we found that while wild-type mice could regenerate up to 75% of their presurgery weight, Casp3^{-/-} mice only recovered about 60% of their mass and Casp7^{-/-} only 57%. BrdU staining determined a 50% reduction in cellular proliferation in both Casp3^{-/-} and Casp7^{-/-} mice when compared with wild type mice post-partial hepatectomy.

These experiments highlighted the importance of apoptosis induction and subsequent activation of caspases-3 and -7 to both the wound healing process and liver regeneration. What are the downstream factors of caspases that are involved? Since the activity of calcium-independent phospholipase A₂ (iPLA₂) is enhanced through cleavage by caspases-3 or -7 and leads to an increase in the synthesis and release of arachidonic acid and lysophosphocholine from apoptotic cells [46–48], we hypothesized that an increase in arachidonic acid could serve as a precursor for prostaglandin E₂ (PGE₂) synthesis, which is known to act as a stimulator of stem cell proliferation [49–51]. Indeed we found that ionizing radiation, along with inducing apoptosis in wild-type MEFs, significantly increased secretion of both arachidonic acid and PGE₂ and that this secretion was greatly diminished in caspase-3 deficient MEFs. Furthermore, western blot analysis confirmed that iPLA₂ cleavage was dependent on caspase-3 and PGE₂ production was dependent on iPLA₂ since shRNA-mediated knockdown of iPLA₂ led to reduced concentrations of PGE₂. Both PGE₂ and activated iPLA₂ were shown to increase keratinocyte proliferation. Figure 1 summarizes this mechanism of caspase-mediated wound healing and tissue regeneration which we have named the “phoenix rising” pathway of tissue regeneration.

Cell death mechanisms and radiotherapy

Radiation therapy is a standard practice in the treatment of cancer with over 50% of patients receiving it at some point during their illness [52]. Ionizing radiation exerts its anticancer effect by reacting with molecular oxygen and water to generate reactive oxygen species (ROS) that can attack deoxyribose in the DNA backbone leading to double strand breaks in DNA [53]. Sublethal doses of radiation can trigger a nuclear DNA damage response, however when the extent of damage becomes too much to repair, cell death mechanisms are initiated [54]. The use of ionizing radiation for cancer therapy must balance the induction of death in malignant cells while limiting destruction to normal tissues.

While radiation treatment has been shown to induce multiple types of cell death, including necrosis [55], autophagy [56], and apoptosis, there are some controversies on the most important mode of cell death during radiotherapy [57]. With regard to apoptosis, a beneficial host response after ionizing radiation may not be so straight forward and may depend more on the cell type and context within the tumor microenvironment [52, 58–60]. Indeed there has been much debate over the contribution of apoptosis per se in radiation therapy since the

1990s. Complicating matters, is that sensitivity to radiation is highly variable depending on the cell type [61] and experimental techniques for discerning apoptosis are just as variable and not always capable of accurately distinguishing apoptotic cell death from other mechanisms.

Irrespective of the mode of death after radiation therapy, there is no denying that radiation is very effective at killing cancer cells. The problem is that a few surviving tumor cells can grow back and cause tumor repopulation and cancer recurrence [62].

Tumor repopulation, a key issue in radiation biology and therapy

Accelerated tumor repopulation after radiation therapy is not a new concept [63–67] and has been noted following chemotherapy as well [68–70]. Early studies carried out by H. Rodney Withers in both mice and later in human clinical trials detailed the occurrence of accelerated tumor repopulation after treatments with ionizing radiation [63, 71] and pioneered the work on modifying radiation treatment regimens to circumvent this effect [72, 73]. It was discovered that in certain cell types the rate of repopulation increases as the duration and intensity of treatment escalates [74, 75], however the molecular mechanisms responsible for this effect were unclear. Much of this earlier work alluded to the fact that repopulation responses were highly dependent on the cell type and dose fractionation. Some studies reported accelerated repopulation occurring only in the late stages of radiation treatment while in other instances, such as in human cervical cancer patients, accelerated repopulation onset time was relatively short [76]. Additionally, squamous cell carcinomas [77], bladder cancer [66], and colorectal carcinomas, were shown to have relatively faster repopulation rates enough to generate concern about utilizing extended treatment times [78].

Some generalizations could be made from the collective results. Having a slower rate of repopulation was noted as a predictor of curability and good prognosis while faster repopulating tumors received less therapeutic benefit from prolonged treatment times. In an attempt to counteract this accelerated growth phenomenon, dose escalation is often necessary, particularly over extended treatment periods [79]. Even though better patient outcomes have been achieved by modifying radiation therapy protocols in attempts to diminish tumor repopulation, it remains a key challenge in radiation therapy as well as chemotherapy. Development of resistance in cancer cells treated with ionizing radiation and the subsequent repopulation by radioresistant cells often results in a more aggressive cancer phenotype with devastating consequences to the patient. Therefore, determining the molecular mechanisms of repopulation and acquired resistance are crucial in the development of more targeted therapies.

As early as in 1956, L. Revesz described how mixing tumor cells killed by radiation with viable tumor cells led to an increase in the growth of the latter in a tumor graft and demonstrated that this occurred through diffusion of a growth stimulating metabolite from the lethally irradiated cells to the viable cells [80]. In a follow up study, he reported that this stimulating effect was not dependent on any one tumor cell type since lethally irradiated foreign mouse tumors and liver tissue suspensions were also effective at promoting growth of the viable cells [81]. The fact that Revesz found that this stimulating factor was released from a variety of dying cell types, gives credence to the existence of an evolutionarily conserved mechanism in which compensatory proliferation of surviving cells is triggered by signals released from the dying cells.

In a more recent study it was shown that in mice an immortal myogenic cell line (C2 C12) formed tumors more rapidly in host muscle that had been irradiated prior to transplant than in nonirradiated control mice [82]. A study by Nguyen et al utilized a mammary chimera mouse model where transplantation of oncogenic Trp53 null epithelium into an irradiated

host also resulted in accelerated tumor growth when compared to transplant into a non-irradiated host [83]. In this case, the effect was attributed to TGF β signaling as reducing TGF β prevented the accelerated tumor growth.

Cell death stimulated tumor repopulation during radiotherapy

Aberrant apoptosis is considered a hallmark of cancer and activation of caspases to induce apoptosis is the prevailing ideology in most cancer treatments [26, 27, 84–86]. However, recent results from our laboratory demonstrated that this model may be over-simplified.

Building on our results on pro-regeneration properties of caspase-3 and -7, we discovered an important role for caspase-3 in tumor cell repopulation during radiotherapy [87]. We found that irradiated caspase-3 deficient mouse embryonic fibroblasts (Casp3^{-/-} MEFs) or caspase-3-attenuated tumor cells were unable to support proliferation of labeled tumor cells *in vitro* or *in vivo* (Figure 2).

As in our previous study showing caspase-3's role in mediating wound healing and tissue regeneration, we again found that caspase-3 could up-regulate arachidonic acid and subsequently PGE₂ production through cleavage of iPLA₂ and that caspase-3 dependent activation of iPLA₂ in dying feeder cells is necessary to promote tumor cell proliferation. Furthermore, arachidonic acid and PGE₂ production were both increased by ionizing radiation in wild-type MEFs and 4T1 tumor cells, but their production was greatly reduced in Casp3^{-/-} MEFs or 4T1shCasp3 cells. Exogenous treatment with PGE₂ also caused tumor cells to grow at a faster rate than untreated cells.

Consistent with our finding of key roles of PGE₂ in tumor cell repopulation, it was previously reported that PGE₂ could activate the β -catenin-Wnt signaling pathway in colon cancer [88, 89]. PGE₂ has also been shown to be necessary for Wnt signaling activation in hematopoietic stem cells, where Wnt signaling controls their self-renewal and bone marrow repopulation [90].

The clinical relevance of our findings was demonstrated in two cohorts of human cancer patients: one head and neck cancer and the other breast cancer (Figure 3). In head and neck cancer patients, high amounts of activated caspase-3 were correlated with high rates of tumor recurrence and in breast cancer patients high caspase-3 levels were correlated with shorter survival times.

Summary

Recent discoveries on the roles of apoptotic caspases in tissue regeneration and in tumor cell repopulation are both surprising and exciting. For cancer therapy, there could be tremendous implications. Efforts to develop agents that activate caspases must now be re-examined. On the other hand, small molecule inhibitors of caspases should now be evaluated for their properties to enhance cancer radiotherapy or chemotherapy. While human clinical trials are lacking in this area, several studies using mouse models indicate blocking apoptosis may increase sensitivity to radiotherapy. For example, in a mouse model of lung cancer, treatment with a caspase-3 inhibitor led to an increase in autophagy and radiosensitivity [91]. In addition, treatment with the pan-caspase inhibitor z-vad, or siRNA directed at caspases-3 and -7, also lead to radiosensitization in breast and lung xenografts which exhibited delayed growth rates and decreases in angiogenesis [92]. Caspase inhibition has also been shown to block VEGF-dependent vascular formation in human umbilical vein endothelial cells with caspase-3 appearing to mediate this apoptosis-dependent angiogenesis [93]. The ability of caspase inhibitors to inhibit angiogenesis may possibly be through modulations in cell adhesion and motility factors that are dependent on caspase-8 activation [94].

The potential to target downstream effectors of caspase activation, such as the prostanoid PGE₂, also exists. While most attempts at modulating PGE₂ production in clinical trials through the use of inhibitors of COX enzymes, such as the NSAIDs, have been applied toward improving cardiovascular and inflammatory disease outcomes, there is growing evidence from preclinical studies that PGE₂ can significantly mediate tumor formation and progression [95].

Another target of caspase-3 activation presents itself in the highly malignant and difficult to treat brain tumor, glioblastoma. In glioblastoma, caspase-3 has been shown to be constitutively activated, but at levels that were insufficient to induce apoptosis. However, this caspase activation was shown to promote migration and invasiveness of glioblastoma cells through caspase-dependent cleavage of the pro-motility protein gelsolin [96].

Furthermore, caspase levels could also be examined as potential prognostic biomarkers. Immunohistochemical analysis of caspase-3 expression in human breast cancer showed a correlation between caspase-3 overexpression and adverse patient outcome [97] a result that is in accord with our own analysis presented in Figure 3. A number of human breast, colon, and pancreatic cancer cell lines and tissues, when compared to normal cells, show constitutive low to moderate levels of caspase-3 and -8 activation despite a lack of apoptotic stimuli [98]. This necessitates looking into the role of elevated caspases in promoting tumor persistence independent of apoptosis induction and the potential adverse outcomes of therapies that seek to increase their activation.

Glossary

TRAIL	TNF-related apoptosis-inducing ligand
TNF	Tumor necrosis factor
FADD	Fas-associated protein with death domain
DISC	Death-inducing signaling complex
Smac/DIABLO	Second mitochondria-derived activator of caspases/Direct IAP binding protein with low pI
IAP	Inhibitors of Apoptosis
BH3	Bcl-2 homology domain 3
BID	BH3 interacting-domain death agonist
DjCLg3	caspase-like gene 3
Wnt3	Wingless-type MMTV integration site family, member 3
Wg	Wingless
Dpp	Decapentaplegic
JNK	c-Jun N-terminal kinase
DIAP1	Drosophila inhibitor of apoptosis 1
Dronc	Drosophila Nedd2-like caspase
Hh	Hedgehog
DrICE	Drosophila interleukin-1 β converting enzyme
Dcp-1	Drosophila caspase 1
p53	Protein 53 or tumor protein 53

dp53	Drosophila protein 53
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
Fluc	Firefly luciferase
MEF	Mouse embryonic fibroblasts
BrdU	Bromodeoxyuridine
iPLA2	Calcium-independent phospholipase A ₂
PGE₂	Prostaglandin E ₂
TGF-β	Transforming growth factor beta
Trp53	Transformation related protein 53
VEGF	Vascular endothelial growth factor
COX	Cyclooxygenase
NSAIDS	Non-steroid anti-inflammatory drugs

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Figure 2.

Critical roles of caspase 3 in cell death-simulated tumor cell proliferation. About 500 4T1Fluc cells were mixed with 2.5×10^5 of lethally irradiated, wild type (right hind legs) or casp3^{-/-} (left hind legs) mouse embryonic fibroblast cells and injected subcutaneously into nude mice. The growth of 4T1Fluc cells were then followed by use of bioluminescence imaging. Please note the logarithmic scale of the Y axis. The error bars represent standard error of the means (n=4). The difference between two groups were highly significant statistically ($P < 0.001$ from day 3 on, one-way ANOVA test).

Figure 3.

Activated caspase 3 (CC-3) in pretreatment biopsies predicts for A) tumor recurrence in head and neck cancer patients who underwent radiotherapy; B) survival in advanced breast cancer patients who underwent surgery and other treatments.