Exp Oncol 2012
34, 3, 277–285

INVITED REVIEW

Experimental ONCOLOGY

APOPTOSIS IN RADIATION THERAPY: A DOUBLE-EDGED SWORD

E.K. Balcer-Kubiczek

University of Maryland School of Medicine, Marlene and Stewart Greenebaum Cancer Center
Department of Radiation Oncology, Baltimore MD 21201, USA

Radiation therapy achieves its therapeutic effects by inducing apoptosis and non-apoptotic cell death. The aim of this focused review is to highlight the aspects of the cell death pathways most relevant to conventional fractionated radiation therapy. I review reports on how our current understanding of the molecular mechanisms of cell death may enable us to revise the four radiobiological principles (reoxygenation, repair of sublethal damage, redistribution of cells in the cell cycle, and repopulation of surviving cells) for radiation treatment with fractionated dose delivery. Apoptosis and non-apoptotic forms of cell death are not represented in the linear quadratic model, which is clinically used to calculate the effects of different total doses, dose per fraction and fraction number on reproductive cell death, a mode of cell death associated with lethal chromosome aberrations. Examples are provided to justify or not a reassessment of the role of apoptosis and non-apoptotic cell death in radiosensitivity, tumor cell proliferation and tumor microenvironment. As our understanding of apoptosis developed at the molecular level, so did our understanding of other forms of cell death, particularly autophagy and to a lesser extent, senescence. The linear quadratic model remains a guide for the treatment planner. The therapeutic clinical roles of apoptosis and non-apoptotic forms of cell death remain to be defined. Their relative importance will probably lie in tumor developmental history related to its type, size and stage. Radiobiological research should focus on the quantitative effects of dose and fractionation on the radiation induction of apoptotic and non-apoptotic types of cell death and the interplay among cell death pathways. This article is part of a Special Issue entitled "Apoptosis: Four Decades Later".

***Key Words*: apoptosis, non-apoptotic cell death, radiation therapy, tumor response, normal tissue response.**

INTRODUCTION

Ionizing radiation has been used clinically for the treatment of a wide range of human cancers for more than 100 years [1]. Radiotherapy reduces the risk of cancer recurrence, promotes tumor control, and improves survival [2]. At least 50% of all cancer patients will receive radiotherapy at some stage during the course of their illness. It is currently used to treat localized solid tumors, such as cancers of the lung, colon/rectum, larynx, thyroid, brain/CNS, breast, prostate, or cervix, and can also be used to treat leukemia and lymphoma [2, 3]. The aim of radiotherapy is to destroy cancer cells with ionizing radiation while limiting the damage to nearby healthy tissue. This aim has been facilitated by innovations in technology and engineering, followed by the computer revolution applied to treatment planning, and the recent development of sophisticated irradiation techniques, including proton and intensity-modulated radiotherapy [4].

Current practice of fractionated radiotherapy, where tumors are irradiated multiple times, usually 30, with small doses per fraction, usually 1.8–2 Gy, over several weeks has its roots in decades of clinical observations and radiobiological research. Collectively, the data indicate that the biologic effects of a given dose depend strongly on the details of how this dose is delivered over time. Fractionation of the radiation dose produces, in most cases, better tumor control while reducing the level of normal tissue damage compared to a single dose. The underlying interpretation has been encapsulated in the four Rs of radiation therapy: repair of radiation-induced damage between fractions, redistribution of cells in the cell cycle, repopulation of the tumor during the treatment period by surviving tumor cells and reoxygenation of hypoxic cells [5]. A fifth R, radiosensitivity expressing a genetic characteristic of cells, has been proposed as a major factor determining radiotherapy individual outcome [6].

There are at least eight forms of cell death that may account for cell killing both normal and tumor tissues [7, 8]. Of those, cell death modalities most relevant to this discussion are apoptosis [9], autophagy [10] (a history of autophagy reviewed in ref. [11]) and the loss of clonogenic survival [12]. Both apoptosis and autophagy, and their crosstalk are important in understanding clonogenic radiosensitivity *in vitro* and *in vivo* [13, 17–19]. The loss of clonogenic survival is the form of cell death quantified by the clonogenic assay, the experimental technique for assessing the fraction of cells dying (or more precisely, of cells surviving) [7, 12]. In the context of radiation biology/oncology cell death is equated with any process that leads to the loss of the proliferative capacity of the cells. Cells that are able to form colonies from a single cell are considered to have survived the treatment. The clonogenic *in vitro* data generally, but not always, agree with tumor response *in vivo* [20–22]. Irradiated cells may also die by mitotic catastrophe, senescence and necrosis [8]. Mitotic catastrophe might not a *bona fide* form

Received: June 8, 2012.
Correspondence: Fax: 410-706-6666
E-mail: ekubicze@umaryland.edu

Abbreviations used: GFP – green fluorescent protein; HIF-1 – hypoxia-inducible factor-1; LC3 – light chain 3 of the microtubule-associated protein 1; a mammalian homolog of autophagy-related protein 8 (Atg8); LQ – the linear quadratic model or formula; NCCD – Nomenclature Committee on Cell Death; PARP-1 – poly(adenine diphosphate-ribose) polymerase-1; TFF1 – trefoil factor 1 (pS2); TFF3 – trefoil factor 3 (ITF); TGF β – transforming growth factor β; TUNNEL – terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate (dUTP) nick end labeling.

of cell death, because cells that experience mitotic catastrophe eventually die by apoptosis or senesce [8, 23]. Senescent cells are considered to be dead reproductively and usually not contributing to radiation response. There is a renewed interest in stress-induced senescence because of a possible relationship between autophagy and senescence in treated tumor cells and the involvement of these two death modalities in tumor dormancy and disease recurrence [24] (reviewed in ref. [25]). In contrast to off-target effects of diffused cytotoxins, radiation-induced "bystander death" is an unclassified and poorly understood type of cell death, perhaps relevant to risk estimation at low radiation dose levels but not effects of typical therapeutic dose levels [26].

Prior reviews dating back more than two decades have addressed the role of apoptosis in determining radiation response [13, 14, 27–31]. The purpose of this review is present a few examples from currently active research with tumors and tumor cells of primarily non-hematological origin that justify a reassessment of the role of apoptosis and non-apoptotic cell death in radiation sensitivity, tumor cell proliferation and tumor microenvironment.

RADIATION SURVIVAL CURVES AND MECHANISMS OF CELL DEATH

Cell death following irradiation is the stochastic effect or "chance effect" which a consequence of the random, statistical nature of damage. Thus, the magnitude of an effect is independent of dose, but the probability of an effect increases with dose. This stochastic nature of cell killing is easily inferred from the shape of survival curves, that is, plots of the logarithm of the percentage of surviving (clonogenic) cells as a function of the dose [12]. The initial portion of survival curve in the low (therapeutic) dose range (<3–4 Gy) can be conveniently described by the linear-quadratic (LQ) formula, which enables radiation biologists and treatment planners to calculate cell killing by different total doses, size of dose fraction, dose rate and fraction number [32].

Implicit in the LQ formula is the assumption that radiation produces two different types of damage: non-repairable damage described by the linear component (~ dose) and repairable damage described by the quadratic component (~ dose^2). Non-repairable damage is synonymous with intrinsic radiosensitivity, because the linear component is invariant with respect to dose delivery variables, such as dose rate or fractionation, but modifiable by genetic background. Well-known examples of genes involved in genetic control of intrinsic radiosensitivity are genes involved in cell-cycle progression and DNA repair (http://sciencepark.mdanderson.org/ labs/wood/dna).

There have been attempts in the 1990s to correlate the incidence of apoptosis with clonogenic cell survival and to factor apoptosis in the LQ formula [14, 15, 27–31]. Several authors proposed that radiation-induced apoptosis contributes only to the linear component of the LQ formula, that is, to intrinsic radiosensitivity of a cell [33–37]. This was primarily based on observations that dose response curves for both apoptosis incidence and non-repairable damage are a linear function of the dose and that apoptosis incidence is independent of how the dose was distributed in time.

It has been now recognized that the above-mentioned direct correlation between sensitivity to the induction of apoptosis and loss of clonogenicity exists only in a limited number of tissues, including thymocytes, spermatogonia, hair-follicle cells, stem cells of the small intestine and bone marrow, and tissues in developing embryos as well tumors arising from these tissues [35, 36, 38–43]. In contrast, other studies with solid tumor models *in vitro* and *in vivo* generally failed to find an association between sensitivity to apoptosis and sensitivity to therapeutic agents including ionizing radiation. For results underlying this conclusion in experimental settings, see refs. [13–15, 27, 28]. Similar results were obtained in clinical settings. The majority of studies found no or negative association between high apoptosis incidence and survival and/or recurrence in cervical, bladder and head and neck patients treated with radiotherapy [44] (and references therein). In addition, a retrospective study of 2739 colorectal cancer patients treated with chemo- and radiotherapy showed no association between apoptosis resistance and treatment failure. However, high apoptosis in a subset of rectal patients correlated less recurrences and/or survival [45] (and references therein).

There have been several explanations of experimental and clinical results described above. As proposed by Brown and colleagues [30, 44], the time period over which apoptosis occurs following irradiation could be different in different cell types. Cells such as thymocytes, lymphocytes, lymphoblasts and stem cells undergo apoptosis shortly after treatment (peaking usually at 3–4 h post-irradiation) and prior to the first division after treatment (also termed an "interphase cell death") [35, 38].In these cells apoptosis incidence generally correlates with clonogenic cell killing. In contrast, this early apoptosis does not occur in epithelial cells and tumors of epithelial or mesenchymal origin. In these cells, apoptosis occurs much later and subsequent to mitosis (also termed a "postmitotic cell death") [28–31, 34, 39]. Late apoptosis does not correlate with clonogenic cell killing and usually does not occur at therapeutic dose levels (<3–4 Gy).

Although the genetic mechanisms of X-ray action on cells were far from understood in the early days of radiation research, scientists realized that the clonogenic assay might not take into account all the consequences of irradiation. For example, they noted that the number of cells in the colonies produced by surviving cells depends on the dose they received: the larger dose, the larger proportion of small colonies that frequently include morphologically-altered cells such as giant and senescent cells [12, 46–53]. Hurwitz and Tolmach [50] and Thompson and Suit

[51] investigated the fate of irradiated cells of different origins using simple imaging tools available in the 1960s. By this approach, they were able to record the division history of specific, initially single cells; that is, whether they divided between observations, produced morphologically normal or altered daughter cells, or disappeared (following their detaching from the substratum). These observations furnished evidence that even "killed" cells can carry out a limited number of divisions and that the average number of divisions of which non-surviving cells are capable before the onset of death varies with a dose and cell type. The elimination of "killed" cells from the colony was attributed to lysis of detached and rounded cells. It is now known that detachment from a solid substrate is one of early morphological features of apoptosis and that apoptotic cells are typically engulfed by surrounding cells, and therefore disappear. The anchorage-dependent mode of cell death by apoptosis induced by abnormal detachment from the extracellular matrix is termed anoikis (from a Greek word meaning "homelessness") [7, 8]. A well-designed study of early and late death processes in irradiated cells [15] and real-time imaging studies in the late 1990s using advanced imaging technologies have refined and extended results on fate of irradiated cells [54–56].

REPOPULATION AND MECHANISMS OF CELL DEATH

Repopulation of tumors, during and after radiation treatment, is considered one of the main reasons for the failure of conventional fractionated radiation therapy, because tumor cell division between fractions may in part compensate for the cell death produced by each fraction [57–60]. The cell population kinetics have been studied experimentally in several animal models as well as be retrospectively by analyzing clinical data [57, 61–63 (reviewed in ref. [58]). It is important to present examples of results from these studies in order to place current research in the proper context. Denekamp [63] and Withers *et al.* [57] showed that tumor repopulation is not evident at the beginning of the treatment and that the process becomes clinically apparent 3–5 weeks after the start of the treatment. This implies that for treatment times shorter than 3–5 weeks tumor proliferation had little effect. Following the lag phase, accelerated repopulation takes place; the term "accelerated repopulation" describes more rapid multiplication surviving clonogens after irradiation than before [57, 61–64]. For treatments longer than 5 weeks, the effect of repopulation is eguivalent to a loss of tumor radiation dose of 0.6–1.3 Gy/day [57, 63, 64].

Although accepted as a process, the mechanisms behind accelerated repopulation and its onset are topics still debated in the literature. One of the possible mechanisms responsible for tumor repopulation is accelerated cancer stem cell division [65, 66]. The cancer stem cell hypothesis proposed that a subset of tumor cells is able to maintain and propagate tumor [67–70]. The term "tumor stem cells" was first used by Makino in 1959 [67] to describe rare tumor cells that are more resistant to chemotherapy than the bulk of tumor cells. The current view is that cancer stem cells originate either from malignant transformation of a normal somatic stem cell or a progenitor (non-stem) cell [65, 66, 68–70]. The possibility of interconversion of tumor stem and non-stem cells and what are key factors in influencing this plasticity are a matter of debate [66, 69–73]. Mechanisms of accelerated repopulation based on the cancer stem cell hypothesis have been proposed by Dörr [74] and more recently revisited by Marcu *et al.* [75] and Pajonk *et al.* [66]. The latter study suggested that radiation damage might recruit quiescent cancer stem cells into the proliferating pool [66]. Other likely mechanisms, named by Dörr [74] "the three As of repopulation", include acceleration of stem cell division, abortive division and asymmetrical loss in stem cell division. Accelerated stem cell division implies a treatment-induced shortening of the cancer stem cell cycle time. Marcu *et al*. [75] modeled post-irradiation accelerated repopulation assuming different cell cycle durations. The authors concluded that accelerated cancer stem cell division is the least likely mechanism responsible for tumor repopulation because it would require a shortening of the cancer stem cell cycle to about 1 h, which is biologically implausible. The third hypothetical mechanism, the loss of asymmetrical division (resulting in two stem cells, instead of one stem and one differentiated cell) remains untested.

An alternative mechanism of tumor repopulation considers non-stem, senescent tumor cells [24, 25, 76]. Cellular senescence could be activated as a part of an adaptive stress response [24, 76–78]. Recent studies demonstrated that the pro-survival function of autophagy (protective autophagy) is required for the efficient execution of the stress-induced senescence program [78–80]. Accordingly, protective autophagy helps stressed tumor cell survive in a setting of increased metabolic demands, mitigate damage and promote recovery of normal functions; alternatively, autophagy helps achieve cellular remodeling associated with senescence by degradation of specific cellular components [78, 81]. Independent regulation of apoptosis and autophagy observed in some cellular settings; in this scenario, inhibiting one death pathway results in activating expressing the other pathway [11, 16, 17]. Crosstalk between the two death pathways was also reported; under this alternative scenario, apoptosis depends on prior autophagy [79, 80]. Gewirtz [25] described a model whereby the functional consequences of protective autophagy and senescence depend on the nature and quantity of the cellular damage. When the damage is extensive such as following a large single dose of radiation (e.g. 20 Gy), autophagy and senescence might be insufficient to maintain cells in a protective state and the majority of irradiated cells die. In contrast, when irradiation is delivered over time, such as during fractionated therapy (typically 6 weeks),

the cells experience progressive but moderate radiation damage after each fraction (about 2 Gy). There are several reports showing that during intra-fraction intervals (typically 24 h), cells are not able to repair completely DNA damage before the application of the next radiation dose induces new DNA damage [13, 15, 82–84]. In two studies [82, 83], accumulation of DNA double strand breaks did not trigger apoptosis *in vivo* and *in vitro* and diverted a fraction of cells into cell cycle exit [82]. The authors hypothesized that a growth arrest phenotype may precede senescence [82].

From the standpoint of radiation therapy concerns, a limitation of the studies by Řezáčová *et al.* [82] and Rűbe *et al.* [83] are that the only times points examined were during fractionated irradiation (up to 5 d in both studies). In contrast, Li *et al.* [85] studied DNA damage over an extended period of up to 21 d post-irradiation; the authors showed two distinct phases of DNA double-strand break induction, an acute phase peaking at 3–5 h during first 24-h post X-irradiation, and a post-acute phase lasting peaking at 5 d during 1–21 d post X-irradiation. In addition, they reported activation of both apoptotic and non-apoptotic pathways in survivors during the second wave of DNA double-strand break induction [85]. These results are consistent with observations from several laboratories [13–17, 22, 23, 41, 46, 47, 51–53], collectively termed "lethal sectoring" [86], which describes the induction of protective and death subroutines in individual survivors. There is no direct evidence that the impairment of autophagy facilitates escape from senescence and reentry of cells to the cell cycle [25]. However, it must be noted that the detailed analysis of patterns of growth of irradiated experimental tumors led Frindel *et al.* [62] to suggest that a proportion of cells exhibiting a growth arrest phenotype "*are in a reversible state and can be stimulated to re-enter division*".

An alternative model has been proposed by Meyn and colleagues [87]. The authors evaluated single dose- and fractionation protocols in experimental tumors and showed that compared to a single dose, fractionated radiation is a more efficient inducer of apoptosis; in fact, a proportion of apoptotic cells was directly correlated with the number of fraction and inversely correlated with tumor growth rates in each radiation protocol. In addition, the authors concluded that the balance between apoptotic death and cell division of survivors after each dose fraction might result in the lag period before the onset of repopulation.

A provocative study by Huang and colleagues [88] provided yet another mechanism of tumor repopulation. They reported that under radiation therapy, dying cells in the tumor mass support the proliferation of other live tumor cells. This work demonstrated that the activation of a key player in apoptotic cell death, caspase-3, in damaged cells is responsible for synthesis and efflux of prostaglandin E_2 .How prostaglandin E_2 stimulate the growth of tumor cells is controversial, because as recently noted both extracellular and intracellular prostaglandin E_2 participates in a receptor- or Bax-mediated apoptotic death, respectively [89]. Connell and Weichselbaum [90] and Lauber *et al.* [91] critically addressed the relevance of work by Huang *et al.* [88] to radiation therapy. Just to highlight one point, Huang *et al.* used one or two large X-ray doses (6–12 Gy) in their experiments [88]. These doses are in the range of doses only used in specialized radiation procedures (for example, proton therapy or stereotactic body radiotherapy) that employ 1–5 fractions delivered over a short period, at most 2 weeks [92]. Because compensatory repopulation starts 3–4 weeks after initiation of radiation therapy, repopulation is not a factor in such types of radiation treatment. In addition, it has been shown that apoptosis-inducing drugs (for example, taxanes or PARP-inhibitors) given prior to radiation therapy significantly reduce tumor growth and volume, compared to radiation therapy alone [23, 93, 94], whereas the opposite effect would be expected based on the study by Huang *et al.* [88].

MICROENVIRONMENT AND MECHANISMS OF CELL DEATH

A solid tumor is a complex system composed of a mass of proliferating tumor cells, a blood vessel network, lymphatic vessels, and a variety of non-tumor cells and molecules all of which contribute to the local microenvironment. The importance of the tumor-specific milieu was recognized more than 120 years ago by Paget who described the concept of "seed and soil" to explain site-specific metastatic dissemination [95]; he concluded that "*although the best work in pathology of cancer is done by those who are studying the nature of the seed*" (cancer cell), the "*observations of the properties of the soil" (optimal milieu for tumor growth) "may also be useful*" [95]. It has been recognized for more than 40 years that interactions between the tumor cell and components of its microenvironment shape and determine the malignancy phenotype. However, how this complex and intertwined tumor system responds to radiation therapy is still poorly understood.

The underlying differences between the physiology of normal and tumor tissues stem from the tumor vasculature [96]. Structurally, tumor vessels are often dilated and leaky. A heterogeneous zonal variability of blood supply within a tumor correlates spatially with metabolic activity and oxygen supply [97]. It has been recently proposed that the tumor vasculature can arise from proliferation of endothelial cells from local, pre-existing vessels (angiogenesis) or by colonization of circulating endothelial and other specific pro-angiogenic cells, mainly myeloid bone marrow-derived cells (vasculogenesis) [98]. Which of the two mechanisms prevails in radiation therapy is a topic still debated in the literature [65, 66, 96–98]. While a more comprehensive discussion of mechanisms of tumor vascularization in naïve and radiation treated tumors is outside the scope of this review, it needs to be mentioned that Kozin *et al.* [99] recently reviewed single-dose effects (12–50 Gy) on a population of endothelial

cells and blood perfusion in preclinical models [99]; the authors concluded that a body of experimental evidence supports endothelial cell-based angiogenesis rather than an alternative mechanism of vasculogenesis proposed by Kioi *et al.* [98].

One group of investigators proposed that the response of tumors to irradiation is affected by the sensitivity of tumor endothelial cells [100, 101]. Garcia-Barros *et al.* reported that the tumor-associated endothelial cells undergo massive and rapid ceramide-mediated apoptosis within few hours after irradiation leading to indirect tumor cell death. There is no independent confirmation of these results, as noted by Kozin *et al.* [99]. Indeed, numerous other studies reported negligible radiation effects on vessel structure and function during a few weeks post-irradiation (see Table I in ref. [99] and references therein). Ogawa *et al.* [102] attributed findings of Garcia-Barros *et al.* to unusual tumor-host relationships in the tumor model they used in [100, 101]. However, note that because of the short experimental time frame, apoptosis of tumor cells that would have occurred at later time points cannot be ruled out. As discussed, apoptosis is not a major contributor of long-term tumor response post-irradiation. An obvious alternative mechanism, not considered by Garcia-Barros *et al.* [100, 101], is direct tumor cell killing by radiation; this can be assessed using the conventional *in vivo* clonogenic assay.

One of consequences of disorganized architecture of tumor vessels is a heterogeneous variation of oxygen within the cell mass ranging from and hypoxic (<0.5% to 1.5% O_2) to normoxic (>1.5% O_2) with median values much lower than normal. The histological studies of human bronchial carcinoma by Thomlinson and Gray were among the first to provide a mechanism for spatially heterogeneous distribution of oxygen concentration in tumors [103]. They postulated that because of their rapid growth, tumor cells are progressively pushed away from vessels beyond the effective diffusion distance (of about 150 μm) thus become hypoxic and eventually necrotic. With minor refinements, this basic mechanism has been validated in cancers of other organs, including the ovary, esophagus, and head and neck [104]. Glucose and nutrient distributions are thought to follow similar patterns to that of oxygen. Consequently, the viable regions of tumor are characterized by variability of oxygen and glucose content in space. In addition, the efficient efflux of hydrogen ions from tumor cells combined with inefficient buffering capacity of tumor interstitial fluid generates extracellular acidosis.

Hypoxia is detrimental to successful radiation therapy because hypoxic cells are typically 2.5 to 3 times radioresistant than normoxic cells (as measured by the clonogenic assay) [104]. Hypoxia is detrimental to chemotherapy because anticancer drugs might not reach the target cells distant from blood vessels [105] and because hypoxia up-regulates genes involved in multidrug resistance [106]. Finally, hypoxia compromises curability by cancer surgery, because the low oxygen environment promotes survival of tumor cells with a more aggressive phenotype, including diminished pro-death mechanisms (for example, apoptosis), enhanced pro-survival mechanisms (for example, switching aerobic to anaerobic energy production or activating protective autophagy and/or senescence) [104–106]. Finally, hypoxia results in a limited response to the presence of cancer cells by the immune system. Thus, hypoxia in solid tumors has a negative impact on the ability of current cancer treatment modalities to control solid tumors.

Landmark studies in the early 1990s demonstrated the operation of a specific oxygen-sensing process controlled by hypoxia-inducible factor-1 (HIF-1) in tumor cells [107]. As a transcription factor, HIF-1 up-regulates more than more than 100 genes coding proteins essential for glucose and iron metabolism, mobility, proliferation, cell survival, immune surveillance, angiogenesis and drug resistance [107]. Together, the consequences of HIF-1 are directed toward maintaining energy production and survival of the tumor in a hostile microenvironment.

Modulation of radiation-induced death pathways by factors associated with the tumor microenvironment (hypoxia, energy depletion and acidosis) is far from completely understood. Several lines of evidence indicate that apoptosis and autophagy co-exist in tumor cells and can be activated as independent pathways, but they are also interconnected processes. For example, both irradiation and hypoxia up-regulate autophagic death and inhibit apoptotic death. However, contributions of these processes to the overall survival depend on the relative magnitude of cellular stresses as well as the cellular context [108–110].

Finally, there is increasing evidence that radiotherapy leads to significant alterations in the tumor microenvironment through the induction of soluble signals (including regulatory proteins, growth factors, cytokines and chemokines) [111, 112]. The most significant among them are survival-regulatory proteins including epidermal growth factor [112], pro-inflammatory cytokines [113], fibroblastic growth factor [114], transforming growth factors α (TGF-α) and β (TGF-β) [79] and trefoil factors 1 (TFF1 and 3 (TFF3) [115]. Unlike fibroblast growth factor, and epithelial growth factors and TGF-α , which are early radiation-induced events, activation of TGF-β in tumor cells is a biphasic event with the second wave of the induction beginning 1 d post-irradiation and persisting for up 2–3 d post-irradiation [116]; the late induction phase may be associated the irradiation-induced oxidative stress [111]. The late extracellular induction of TGF-β has a tissue-wide, broad spectrum of cellular consequences including growth arrest, differentiation, migration, invasion, angiogenesis, evasion of the immune system, and apoptosis [117]. In addition, the TGF-β induction in fractionated radiation may lead to *de novo* interactions between microenvironmental factors and tumor cells, and between different microenvironment factors with each dose delivery, thus per-

petuating its bioactivity during radiation therapy [111]. Trefoil factors, TFF1 and TFF3, represent a distinct class of tumor suppressor genes, whose downstream functions in irradiated cells remain yet to be elucidated. However, recent studies shed some light on the nature of the cellular and molecular events targeted by TFF signaling [118–122]. Together, these results indicate that the secreted TFF1 and TFF3 proteins have anti-apoptotic, anti-inflammatory and, paradoxically, anti-proliferative effects on the tumor and its microenvironment. Whether and to what degree, the action of TFF proteins might counterbalance the effects of growth factors and other soluble proteins remains unknown. However, clinical relevance of TFF1 and TFF3 to radiation therapy can be established based on two effects. First, both genes are activated in a p53-independent fashion [123]; p53 is the most frequently mutated gene in human cancers [124]. Secondly, both genes display the unique coordinate, delayed and persistent expression pattern in irradiated cells [115, 121, 123]. Thus, trefoil factors might exert long-lasting protective effects on normal tissues outside the radiation treatment volume; examples include the salivary gland, heart, lung, colon, small intestine and prostate, because these normal tissues are unavoidably irradiated in the course radiotherapy of head and neck, breast, lung and prostate cancers.

CONCLUDING THOUGHTS

Despite the enormous importance of the discovery of molecularly controlled death pathways, the contribution of apoptosis, autophagy and senescence to radiation induced cell death as measured long-term in solid tumors (by clonogenic assays *in vitro* and *in vivo*) remains unclear.

One reason might be the frequent use of the apoptosis-necrosis paradigm or, more recently, the autophagy-senescence-necrosis paradigm to describe total cell killing death following irradiation. As noted in this review and previously by others (notably by Steel [27], Brown and Attardi [30]) most of such studies assessed radiation apoptotic and non-apoptotic effects at an early fixed time after a single large dose (usually ~10 Gy). Thus, future radiobiological research should focus on the quantitative (rather than qualitative) effects of dose, fractionation and time on the induction of apoptotic and non-apoptotic types of cell death. At present, the published data are too fragmentary even to conclude whether or not there is a dose threshold for the induction of different modes of cell death.

The second observation is the often-imprecise and confusing classification of cell death in the literature. For example, the term "apoptosis" is frequently misapplied in the context of cell death by radiation. The Nomenclature Committee on Cell Death (NCCD) published the guidelines in 2008 and 2012 on the use of cell death terminology [7, 8], but those are usually not followed [125]. The NCCD reports emphasized the importance of the biochemical features rather than the commonplace reliance on morphological features. As discussed by Bucur *et al.* [125], the same techniques used to detect apoptosis can also detect necrosis (examples include microscopic observations of DNA fragmentation, TUNEL and Annexin V staining). Popular autophagy detection methods that rely on solely the redistribution of GFP-LC3 fusion proteins into vesicular structures are not considered sufficient for diagnosis [8].

Thirdly, because of tumor heterogeneity in a single patient and phenotypic variations among patients undergoing radiation therapy for the same clinically defined disease, it would be important to assess whether and how the different death types within the tumor (and among patients) might evolve in the course of treatment. Radiation affects multiple facets of tumor cell physiology. Consequently, it could be expected that different cell death mechanisms are not mutually exclusive but rather operate in side-by-side or, conversely, overlap albeit to a variable degree and several characteristics might be displayed at the same time and most likely in a dose-dependent manner. The crosstalk between pro-survival and pro-death pathways and the activation of yet unknown backup pathways add to the complexity of how the cell eventually dies.

ACKNOWLEDGMENT

I thank my colleagues, Dr. George H. Harrison and Dr. Juong Rhee, in the Department of Radiation Oncology, University of Maryland School of Medicine for reading the manuscript and providing valuable comments.

REFERENCES

1. Bernier J, Hall EJ, Giaccia A. Radiation oncology: a century of achievements. Nat Rev Cancer 2004; **4**: 737–47.

2. Lawrence TS, Ten Haken RK, Giaccia A. Principals of Radiation Oncology. In: DeVita VT, Lawrence TS, Rosenberg SA, eds. Cancer Principals and Practice of Oncology, 8th ed. Philadelphia: Lippincott Williams and Wilkins, 2008.

3. Tobias JS. Risk management and radiotherapy for cancer. Clin Risk 1992; **6**: 13–6.

4. Balcer-Kubiczek EK. Radiation Oncology. In: M Schwab, ed. Encyclopedia of Cancer, 4th ed. Heidelberg: Springer-Verlag, 2011.

5. Withers HR. The four R's of radiotherapy. Adv Radiat Biol 1975; **5**: 241–7.

6. Steel GG, McMillan TJ, Peacock JH. The 5Rs of radiobiology. Int J Radiat Biol 1989; **56**: 1045–8.

7. Kroemer G, Galluzzi L, Vandenabeele P, ***et al***. Classification of cell death: Recommendations on the Nomenclature Committee on Cell Death 2009. Cell Death Differ 2009; **16**: 3–11.

8. Galluzzi L, Vitale I, Abrams JM, ***et al***. Molecular definition of cell death subroutines: Recommendations on the Nomenclature Committee on Cell Death 2012. Cell Death Differ 2012; **19**: 107–20.

9. Kerr JF, Wylie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 1972; **28**: 239–57.

10. de Duve C, Wattiaux R. Functions of lysosomes. Annu Rev Physiol 1966; **28**: 435–92.

11. Klionsky DJ. Autophagy: from phenomenology to molecular understanding in less than a decade. Nat Rev Mol Cell Biol 2007; **8**: 931–7.

12. Puck TT, Marcus P. Action of X-rays on mammalian cells. J Exp Med 1956; **103**: 653–6.

13. Dunne AL, Price ME, Mothersill C, ***et al***. Relationship between clonogenic radiosensitivity, radiation-induced apoptosis and DNA damage/repair in human colon cancer cells. Br J Cancer 2003; **89**: 2277–83.

14. Held KD. Radiation-induced apoptosis and its relationship to loss of clonogenic survival. Apoptosis 1997; **2**: 265–82.

15. Williams JR, Zhang YG, Zhou HM, ***et al***. Genotype-dependent radiosensitivity: Clonogenic survival, apoptosis and cell-cycle distribution. Int J Radiat Biol 2008; **84**: 151–64.

16. Apel A, Herr I, Schwarz H, ***et al***. Blocked autophagy sensitizes carcinoma cells to radiation therapy. Cancer Res 2008; **68**: 1485–94.

17. Gerwitz DA, Hilliker ML, Wilson EN. Promotion of autophagy as a mechanism for radiosensitization of breast cancer cells. Radiother Oncol 2009; **92**: 323–8.

18. Lemasters JJ, Nieminen AL, Qian T, ***et al***. The mitochondrial permeability transition in cell death: a common mechanism in necrosis, apoptosis and autophagy. Biochim Biophys Acta 1998; **1366**: 177–96.

19. Long JS, Ryan KM. New frontiers in promoting tumor cell death: targeting apoptosis, necroptosis and autophagy. Oncogene 2012 [Epub ahead of print].

20. Hewitt HB, Wilson CW. A survival curve for mammalian leukeamia cells irradiated *in vivo* (implications for the treatment of mouse leukeamia by whole body irradiation). Br J Cancer 1969; **13**: 373–91.

21. Withers HR. The dose-survival relationship for irradiation of epithelial cells of mouse skin. Br J Radiol 1967; **40**: 187–94.

22. Waldman T, Zhang Y, Dillehay L, ***et al***. Cell cycle arrest versus cell death in cancer therapy. Nat Med 1997; **3**: 1034–6.

23. Balcer-Kubiczek EK, Attarpour M, Jiang J, ***et al***. Cytotoxicity of docetaxel (Taxotere®) used as a single agent and combination with radiation in human gastric, cervical and pancreatic cancer cells. Chemother 2006; **52**: 231–40.

24. Uhr JW, Pantel K. Controversies in clinical cancer dormancy. PNAS 2011; **108**: 12396–400.

25. Gawirtz DA. Autophagy, senescence and tumor dormancy in cancer therapy. Autophagy 2009; **5**: 1232–4.

26. Mothersill C, Seymour CB. Radiation-induced bystander effects — implication for cancer. Nat Rev Cancer 2004; **4**: 158–64.

27. Steel GG. The case against apoptosis. Acta Oncol 2001; **40**: 968–75.

28. Brown JM, Wouters BG. Apoptosis, p53, and tumor sensitivity to anticancer agents. Cancer Res 1999; **59**: 1391–9.

29. Verheij M, Bartelink H. Radiation-induced apoptosis. Cell Tissue Res 2000; **301**: 133–42.

30. Brown JM, Attardi LD. The role of apoptosis in cancer development and treatment response. Nat Rev Cancer 2001; **5**: 231–7.

31. Dewey WC, Ling CC, Meyn RE. Radiation-induced apoptosis: Relevance to radiotherapy. Int J Radiat Oncol Biol Phys 1995; **33**: 781–96.

32. Fowler JF. The linear-quadratic formula and progress in fractionated radiotherapy. Br J Radiol 1989; **62**: 679–94.

33. Ling CC, Chen CH, Lin WX. Apoptosis induced at different dose rates: implication for the shoulder region of cell survival curves. Radiother Oncol 1994; **32**: 129–36.

34. Abend M, Rhein A, Gilbertz KP, ***et al***. Correlation of micronucleus and apoptosis assays with reproductive cell death. Int J Radiat Biol 1995; **67**: 315–26.

35. Olive PL, Frazer G, Banath JP. Radiation-induced apoptosis measured in the TK6 human B lymphoblast cells using the comet assay. Radiat Res 1993; **136**: 130–6.

36. Hendry JH, Potten CS, Chadwick C, ***et al***. Cell death (apoptosis) in the mouse small intestine after low doses: effects of low dose rate, 14.7 MeV neutrons and 600 MeV (maximum energy) neutrons. Int J Radiat Biol 1982; **42**: 611–20.

37. Ling CC, Chen H, Fuks Z. An equation for the dose response of radiation-induced apoptosis: possible incorporation with the LQ model. Radiother Oncol 1994; **33**: 17–22.

38. Lowe SW, Schmidt EM, Smith SW, ***et al***. p53 is required for radiation-induced apoptosis in mouse thymocytes. Nature 1993; **362**: 647–9.

39. Potten CS, Grant HK. The relationship between ionizing radiation-induced apoptosis and stem cells in the small and large intestine. Br J Cancer 1998; **78**: 993–1003.

40. Komarova EA, Chernov MV, Franks R, ***et al***. Transgenic mice with p53-responsive lacZ: p53 activity varies dramatically during normal development and determines radiation and drug sensitivity *in vivo*. EMBO J 1997; **16**: 1391–400.

41. Song S, Lambert PF. Different responses of epidermal and hair follicular cells to radiation correlates with distinct patterns of p53 and p21 induction Am J Pathol 1999; **155**: 1121–7.

42. Potten CS. What is an apoptotic index measuring? A commentary. Br J Cancer 1996; **74**: 1743–8.

43. Baumann M, Krause M, Hill R. Exploring the role of cancer stem cells in radioresistance. Nat Rev Cancer 2008; **8**: 545–54.

44. Brown M, Wilson G. Apoptosis genes and resistance to cancer therapy. What do the experimental and clinical data tell us? Cancer Biol Ther 2003; **2**: 477–90.

45. de Bruin EC, Medema JP. Apoptosis and non-apoptotic deaths in cancer development and treatment response. Cancer Treatment Rev 2008; **34**: 737–49.

46. Tolmach LJ. Growth patterns of X-irradiated HeLa cells. Ann NY Acad Sci 1961; **95**: 743–57.

47. Elkind MM, Han A, Voltz KW. Radiation response of mammalian cells grown in culture. IV. Dose dependence of division delay and postirradiation growth of surviving and non-surviving Chinese hamster cells. J Natl Cancer Inst 1963; **30**: 705–21.

48. Dewey WC, Humphrey RM, Cork A. Comparison of cell-multiplication and colony-formation as criterion for radiation damage in cells growth *in vitro*. Int J Radiat Biol 1963; **6**: 463–71.

49. Michalowski A. A critical appraisal of clonogenic survival assays in the evaluation of radiation damage of normal tissue. Radiother Oncol 1984; **1**: 241–6.

50. Tolmach LJ, Marcus PJ. Development of X-ray induced HeLa giant cells. Exp Cell Res 1960; **20**: 350–60.

51. Hurwitz C, Tolmach LJ. Time lapse cinematographic studies of X-irradiated HeLa S3 cells. I. Cell progression and cell disintegration. Biophys J 1969; **9**: 607–33.

52. Thompson LH, Suit HD. Proliferation kinetics of X-irradiated mouse L cells studied with time-lapse photography. Int J Radiat Biol 1969; **15**: 347–62.

53. Balcer-Kubiczek EK, Yin J, Harrison GH, ***et al***. p53 mutational status and survival of human breast cancer MCF-7 cell variants after exposure to X rays and neutrons. Radiat Res 1995; **142**: 256–62.

54. Vidair CA, Chen CH, Ling CC, ***et al***. Apoptosis induced by X-irradiation of REC-myc cells is postmitotic and not predicted by the time after irradiation or behavior of sister cells. Cancer Res 1996; **56**: 4116–8.

55. Forrester HB, Vidair CA, Albright N, ***et al***. Utilizing computerized video-time lapse for quantifying cell death of x-irradiated rat embryo cells transfected with c-myc or c-Ha-ras. Cancer Res 1999; **59**: 931–9.

56. Endlich B, Radford IR, Forrester BH, ***et al***. Computerized video time-lapse microscopy studies of ionizing

radiaton-induced rapid-interphase and mitosis-related apoptosis in lymphoid cells. Radiat Res 2000; **153**: 36–48.

57. Withers HR, Taylor JM, Maciejewski B. The hazard of accelerated tumour clonogen repopulation during radiotherapy. Acta Oncol1988; **27**: 131–46.

58. Kim JJ, Tannock JF. Repopulation of cancer cells during therapy: an important cause of treatment failure. Nat Rev Cancer 2005; **5**: 516–25.

59. Kupelian P. Radiotherapy: Effect of treatment interruption in prostate cancer. Nat Rev Clin Ocol 2009; **6**: 312–3.

60. Bese NS, Sut PA, Ober A. The effect of treatment interruptions in the postoperative irradiation of breast cancer. Oncology 2005; **69**: 214–23.

61. Malaise E, Tubiana M. Croissance des cellules d'un fibrosarcome experimental irradié chez la souris C3H. Compt Rend Acad Sci 1966; **263**: 292–5.

62. Frindel E, Malaise EP, Alpen E, ***et al***. Kinetics of cell proliferation of an experimental tumor. Cancer Res 1967; **27**: 1122–31.

63. Denekamp J. Changes in the rate of repopulation during multifraction irradiation of mouse skin. Br J Radiol 1973; **46**: 381–7.

64. Roberts SA, Hendry JH. The delay before onset of accelerated tumor cell repopulation during radiotherapy: a direct maximum-likelihood analysis of a collection of worldwide tumour-control data. Radiother Oncol 1993; **29**: 69–74.

65. Baumann M, Krause M, Hill R. Exploring the role of cancer stem in radioresistance. Nat Rev Cancer 2008; **8**: 545–54.

66. Pajonk F, Vlashi E, McBride WH. Radiation resistance of cancer stem cells: the 4 Rs of radiobiology revisited. Stem Cells 2010; **28**: 639–48.

67. Makino S. The role of tumor stem-cells in re-growth of the tumor following drastic applications. Acta Unio Int Contra Cancrum 1959; **15** (suppl 1): 196–8.

68. Reya T, Morrison SJ, Clarke MF, ***et al***. Stem cells, cancer, and cancer stem cells. Nature 2001; **414**: 105–11.

69. Mimeault M, Hauke R, Mehta PP, ***et al***. Recent advances in cancer stem/progenitor cell research: therapeutic implications for overcoming resistance to the most aggressive cancers. J Cell Mol Med 2007; **11**: 981–1011.

70. Moore N, Lyle S. Quiescent, slow cycling stem cell populations in cancer: a review of the evidence and discussion of significance. J Oncol 2011; **2011**: 396076.

71. Gupta PB, Chaffer CL, Weiberg RA. Cancer stem cells: mirage or reality. Nat Med 2009; **15**: 1010–2.

72. Kelly PN, Dakic A, Adams JM, ***et al***. Tumor growth need not be driven by rare cancer stem cells. Science 2007; **317**: 337.

73. Tubiana M, Koscielny S. On clonogenic tumour cells and metastasis-forming cells. Nat Rev Cancer 2008; **8**: 990.

74. Dörr W. Three A's of repopulation during fractionated irradiation of squamous epithelia: asymmetry loss, acceleration of stem cell division and abortive divisions. Int J Radiat Biol 1977; **72**: 635–43.

75. Marcu L, Lyons AB, Bezak E, ***et al***. The onset of tumour repopulation after radiotherapy in theoretical and *in vitro* models. Asian J Cancer 2004; **3**: 167–71.

76. Vessella RL, Pantel K, Mohla S. Tumor cell dormancy. An NCI workshop report. Cancer Biol Ther 2007; **6**: 1496–504.

77. Lu Z, Luo RZ, Lu Y, ***et al***. The tumor suppressor gene ARHI regulates autophagy and tumor dormancy in human ovarian cancer cells. J Clin Invest 2008; **118**: 3917–29.

78. Young AR, Narita M, Ferreira M, ***et al***. Autophagy mediates the mitotic senescence transition. Genes Dev 2009; **23**: 798–803.

79. Maiuri MC, Zalckvar E, Kimchi A, ***et al***. Self-eating and self-killing: cross-talk between autophagy and apoptosis. Nat Rev Mol Cell Biol 2007; **8**: 741–52.

80. Vicencio JM, Galluzzi L, Tajeddine N, ***et al***. Senescence, apoptosis or autophagy? When a damaged cell must decide its path — a mini review. Gerantology 2008; **54**: 92–9.

81. White E, Lowe SW. Eating to exit: autophagy enabled senescence revealed. Genes Dev 2009; **23**: 784–7.

82. Řezáčová M, Rudolfovă G, Tichy A, ***et al.*** Accumulation of DNA damage and cell death after fractionated irradiation. Radiat Res 2011; **175**: 708–18.

83. Rűbe CE, Fricke A, Wendorf J, ***et al***. Accumulation of DNA double-strand breaks in normal tissues after fractionated irradiation. Int J Radiat Oncol Biol Phys 2010; **76**: 1206–13.

84. Takahashi M, Takakura K, Furusawa Y. Comparison of the kinetics of radiation induced apoptosis in DT40 cells irradiated with low and high doses of X rays. Radiat Res 2010; **173**: 645–50.

85. Li W, Li F, Huang Q, ***et al***. Quantitative noninvasive imaging of radiation-induced DNA double-strand breaks *in vivo*. Cancer Res 2011; **71**: 4130–7.

86. Sasaki H. Lethal sectoring, genomic instability, and delayed division in HeLa S3 cells surviving alpha- or X-irradiation J Radiat Res (Tokyo) 2004; **45**: 497–508.

87. Meyn RE, Stephens LC, Hunter NR, ***et al***. Reemergence of apoptotic cells between fractionated doses in irradiated murine tumors. Int J Radiat Oncol Biol Phys 1994; **30**: 619–24.

88. Huang Q, Li F, Liu X, ***et al***. Caspase 3-mediated stimulation of tumor repopulation during cancer radiotherapy. Nat Med 2011; **17**: 860–6.

89. Lalier L, Pedelaborde F, Braud C, ***et al***. Increase in intracellular PGE_2 induces apoptosis in Bax-expressing colon cancer cell. BMC Cancer 2011; **11**: 153.

90. Connell PP, Weichselbaum RR. A downside to apoptosis in cancer therapy? Nat Med 2011; **17**: 780–2.

91. Lauber K, Munoz L, Berens C, ***et al***. Apoptosis induction and tumor cell repopulation: The yin and yang of radiotherapy. Radiat Oncol 2011; **6**: 176.

92. Song CW, Park H, Griffin RJ. Radiobiology of stereotactic radiosurgery and stereotactic body radiation therapy. In: Levitt SH, Purdy JA, Perez CA, eds. Technical basis of radiation therapy: Practical clinical applications. Berlin Heidelberg: Springer-Verlag, 2012.

93. Milas L, Milas MM, Mason KA. Combination of taxanes with radiation: preclinical studies. Semin Radiat Oncol 1999; **9**: 12–26.

94. Rouleau M, Patel A, Hendzel MJ, ***et al***. PARP inhibition: PARP1 and beyond. Nat Rev Cancer 2010; **10**: 293–301.

95. Paget S. The distribution of secondary growths in cancer of the breast. Lancet 1889; **1**: 521–73.

96. Goal S, Hon-Kit Wong A, Jain RK. Vascular normalization as a therapeutic strategy for malignant and nonmalignant disease. Cold Spring Harb Perspect Med 2012; **2**: a006486.

97. Jain RK. Normalization of tumor vasculature: An emerging concept in antiangiogenic therapy. Science 2005; **307**: 58–62.

98. Kioi M, Vogel H, Schultz G, ***et al***. Inhibition of vasculogenesis, but not angiogenesis, prevents the recurrence of glioblastoma after irradiation in mice. J Clin Invest 2010; **130**: 694–705.

99. Kozin SV, Duda DG, Munn LL, ***et al***. Neovascularization after irradiation: What is the source of newly formed vessels in recurring tumors? J Natl Cancer Inst 2012; **104**: 899–905.

100. Garcia-Barros M, Paris F, Cordon-Cardo C, ***et al***. Tumor response to radiotherapy regulated by endothelial cell apoptosis. Science 2003; **300**: 1155–9.

101. Garcia-Barros M, Thin TH, Maj J, ***et al***. Impact of stromal sensitivity on radiation response of tumors implanted in SCID hosts revisited. Cancer Res 2010; **70**: 8179–86.

102. Ogawa K, Boucher Y, Kashiwagi, ***et al***. Influence of tumor cell and stroma sensitivity on tumor response to radiation. Cancer Res 2007; **67**: 4016–21.

103. Thomlinson RH, Gray LH. The histological structure of some human lung cancers and possible implications for radiotherapy. Br J Cancer 1955; **9**: 539–49.

104. Rockwell S, Dobrucki IT, Kim EY, ***et al***. Hypoxia and radiation therapy: Past history, ongoing research, and future promise. Curr Mol Med 2009; **9**: 442–58.

105. Brown JM, Wilson WR. Exploiting tumour hypoxia in cancer treatment. Nat Rev Cancer 2004; **4**: 437–47.

106. Rohwer N, Cramer T. Hypoxia-mediated drug resistance: Novel insights on the functional interaction of HIFs and death pathways. Drug Resist Updat 2011; **14**: 191–201.

107. Semenza GL. Targeting HIF-1 for cancer therapy. Nat Rev Cancer 2003; **3**: 721–32.

108. Hunter A, Hendrikse A, Renan M, ***et al***. Does the tumor microenvironment influence radiation-induced apoptosis? Apoptosis 2006; **11**: 1727–35.

109. Moretti L, Attia A, Kim KW, ***et al***. Crosstalk between Bak/Bax and mTOR signalling regulates radiation-induced autophagy. Autophagy 2007; **3**:142–4.

110. Amaravadi RK, Lippincott-Schwartz J, Yin XM, ***et al***. Principles and current strategies for targeting autophagy for cancer treatment. Clin Cancer Res 2011; **17**: 654–66.

111. Barcellos-Hoff MH, Park C, Wright EG. Radiation and the microenvironment — tumorigenesis and therapy. Nat Rev Cancer 2005; **5**: 867–75.

112. Dent P, Yacoub A, Fisher PB, ***et al***. MAPK pathways in radiation response. Oncogene 2003; **22**: 5885–96.

113. McBride WH, Chiang CS, Olson JL, ***et al***. A sense of danger from radiation. Radiat Res 2004; **162**: 1–19.

114. Fuks Z. Modulation of the radiation response *in vitro* and *in vivo* by basic fibroblast growth factor Proc Am Assoc Cancer Res 1994; **35**: 709–10.

115. Balcer-Kubiczek EK, Harrison GH, Xu JF, ***et al***. Coordinate late expression of trefoil peptide genes (pS2/TFF! and ITF/TFF3) in human breast, colon, and gastric tumor cells exposed to X-rays. Mol Cancer Ther 2002; **1**: 405–25.

116. Rödel F, Schaller U, Schultze-Mosqau S, ***et al***. The induction of TGF-beta and NF-kappaB parallels a biphasic time course of leukocytes/endothelial cell adhesion following low-dose X-irradiation. Strahlenther Oncol 2004; **180**: 194–200.

117. Blobe GC, Schiemann WP, Lodish HF. Role of transforming growth factor β in human disease. New Engl J Med 2000; **342**: 1350–8.

118. Buache E, Etique N, Alpy F, ***et al***. Deficiency in trefoil factor 1 (TFF1) increases tumorigenecity of human breast cancer cells and mammary tumor development in TFF1-knockout mice. Oncogene 2011; **30**: 3261–73.

119. Bossenmeyer-Pourié C, Kannan R, Ribieras S, ***et al***. The trefoil factor 1 participates in gastrointestinal cell differentiation by delaying G1-S phase transition and reducing apoptosis. J Cell Biol 2002; **157**: 761–70.

120. Buron N, Guerry L, Creuzot-Garcher C, ***et al***. Trefoil factor 1-induced protection of conjunctival cells from apoptosis at premitochondrial and postmitochondrial levels. Invest Ophthal Visual Sci 2008; **49**: 3790–8.

121. Balcer-Kubiczek EK, Garofalo MC. Molecular targets in gastric cancer and apoptosis. In: Chen GG, Lai PBS, eds. Apoptosis in Carcinogenesis and Chemotherapy. Hong-Kong: Springer-Verlag, 2009.

122. Konoshita K, Taupin DR, Podolsky D. Distinct pathways of cell migration and antiapoptotic response to epithelial injury: Structure-function analysis of human intestinal trefoil factor. Mol Cell Biol 2000; **20**: 191–8.

123. Balcer-Kubiczek EK, Zhang XF, Harrison GH, ***et al***. Delayed expression of hppS2 and prolonged expression of CIP1/WAF1/SDI1 in human tumour cells irradiated with x-rays, fission neutrons or 1 GeV/nucleon Fe Ions. Int J Radiat Biol 1999; **75**: 529–41.

124. Kinzler KW, Vogelstein B. Gatekeepers and caretakers. Nature 1998; **386**: 761–63.