OncoImmunology 2:10, e26536; October 2013; © 2013 Landes Bioscience

Immunogenic cell death in radiation therapy

Lorenzo Galluzzi[1,2,3,*,†], Oliver Kepp[3,4,5], and Guido Kroemer[2,3,4,5,6,*,†]

[1]Gustave Roussy; Villejuif, France; [2]Université Paris Descartes/Paris V; Sorbonne Paris Cité; Paris, France;
[3]Equipe 11 labellisée par la Ligue Nationale contre le Cancer; Centre de Recherche des Cordeliers; Paris, France; [4]INSERM, U848; Villejuif, France;
[5]Metabolomics and Cell Biology Platforms; Gustave Roussy; Villejuif, France; [6]Pôle de Biologie; Hôpital Européen Georges Pompidou; AP-HP; Paris, France

[†]These authors share senior co-authorship.

Keywords: ATP, caspases, chemotherapy, immunogenic cell death, radiotherapy, tumor-infiltrating lymphocytes

Radiation therapy has been extensively employed throughout the last century to treat individuals affected by multiple types of tumors, with a variable degree of success. Current estimates indicate indeed that at least 50% of cancer patients all confounded have been or will be exposed to ionizing irradiation, either as a standalone intervention or combined with chemo- and/or immunotherapeutic regimens, be it with curative or palliative intents.[1-3] The consistent technological advances achieved in the past few decades have significantly broadened the clinical benefits of radiation therapy. Thus, ionizing irradiation can nowadays be selectively targeted to malignant lesions while causing limited side effects.[1,3] Such adverse events, which generally stem from the unavoidable irradiation of healthy tissues, reflect the preferential toxicity of irradiation for highly-proliferating cells, and often (though not always) resolve within a few weeks from the interruption of treatment.[4] In addition, radiation therapy has been associated with a small but quantifiable increase in the risk of contracting a second cancer later in life, especially among individuals that have received ionizing irradiation as children or teenagers.[5]

For a long time, the antineoplastic effects of radiation therapy were entirely attributed to its ability to transfer high amounts of energy to irradiated tissues, resulting in some extent of direct macromolecular damage as well as in the overproduction of cytotoxic factors including reactive oxygen species (ROS).[3] Thus, cells exposed to ionizing irradiation either undergo a permanent proliferative arrest known as cell senescence or succumb to the activation of the DNA damage response, most often (though not exclusively) triggering the intrinsic pathway of apoptosis.[6,7] Nowadays, it has become clear that cancer cell-intrinsic mechanisms cannot account for the therapeutic activity of irradiation in vivo. Accumulating evidence suggests indeed that cancer cells succumb to radiation therapy while (1) releasing ROS and other cytotoxic molecules that may kill neighboring cells (local bystander effects),[8,9] and/or (2) eliciting a tumor-specific immune response that exert antineoplastic effects at the systemic level (long-range bystander, out-of-field or abscopal effects)[10-12] (**Fig. 1**). Of note, abscopal-like reactions have been documented not only in mice, but also in sporadic cancer patients treated with radiation therapy.[3,11]

In fact, radiation therapy appears to promote a functionally peculiar type of apoptosis that has been named "immunogenic cell death" (ICD).[13,14] Thus, contrarily to cells that undergo conventional forms of apoptosis, cancer cells exposed to ionizing irradiation die while emitting a specific combination of signals that stimulates antigen-presenting cells to cross-prime antigen-specific adaptive immune responses.[13,14] At least in mice, ICD obligatorily impinges on a few key cell death-associated processes, including (1) the exposure of the endoplasmic reticulum chaperone calreticulin on the cell surface; (2) the autophagy-dependent secretion of ATP; and (3) the release of the non-histone chromatin-binding protein high mobility group box 1 (HMGB1).[13,14]

The cancer cell-intrinsic and extrinsic mechanisms that underlie the emission of these immunogenic signals by dying cancer cells have just begun to emerge,[15-18] and macroautophagy (hereafter referred to as autophagy) appears to play a central role in this setting.[19-22] Indeed, the pharmacological or genetic inhibition of autophagy has been shown to abolish the ability of cancer cells undergoing ICD to vaccinate syngeneic mice against the subsequent inoculation of living cells of the same type.[20,22] This effect has been mechanistically linked to the fact that autophagy is required for the optimal release of ATP during ICD, possibly because it contributes to the preservation of vesicular ATP stores, at least in the initial stages of the lethal process.[21]

Autophagy actually represents an evolutionarily conserved mechanism of adaptation to stress that operates both in "steady-state" conditions, hence favoring the maintenance of intracellular homeostasis,[23] and in response to a wide panel of homeostatic perturbations, including nutritional, chemical and physical cues (notably, ionizing irradiation).[24] In this latter scenario, autophagy exerts major cytoprotective effects, as demonstrated by a consistent amount of literature reporting that the pharmacological or genetic inhibition of essential autophagic factors accelerate, rather than inhibit, the cellular demise.[24] Based on these premises, the pharmacological inhibition of

*Correspondence to: Lorenzo Galluzzi; Email: deadoc@vodafone.it; Guido Kroemer; Email: kroemer@orange.fr
Submitted: 09/11/2013; Accepted: 09/11/2013
Citation: Galluzzi L, Kepp O, Kroemer G. Immunogenic cell death in radiation therapy. OncoImmunology 2013; 2:e26536; http://dx.doi.org/10.4161/onci.26536

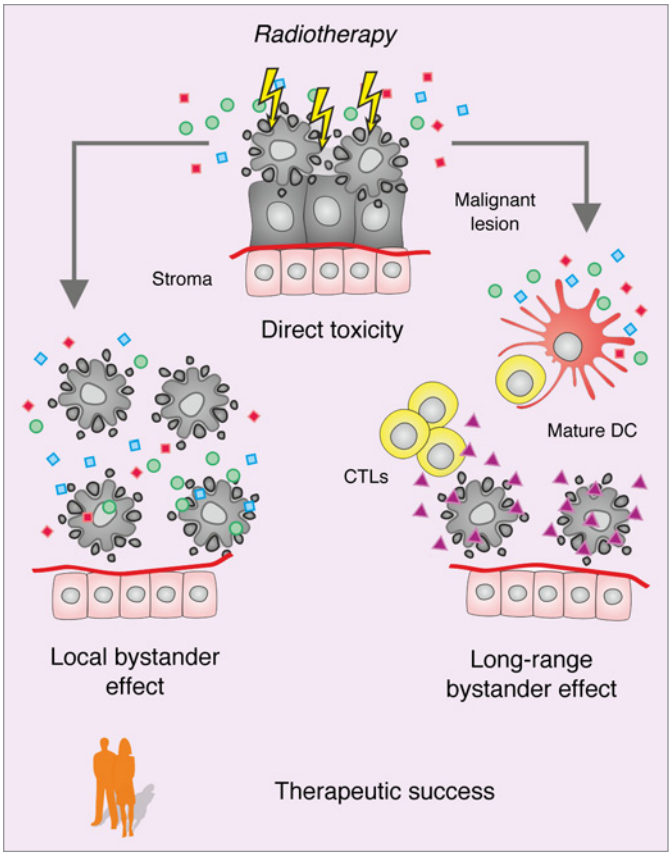

Figure 1. Immunogenic cell death in radiation therapy. Irradiated cancer cells generally undergo a permanent proliferation arrest known as cell senescence or succumb to mitochondrial apoptosis upon the activation of the DNA damage response. As they die, these cells release potentially cytotoxic factors such as reactive oxygen species, which may promote the demise of neighboring, non-irradiated or radioresistant cells (local bystander effect). In immunocompromised hosts, these 2 mechanisms account for most, if not all, the therapeutic efficacy of ionizing irradiation. When neoplastic cells succumb to radiation therapy, they also emit a specific combination of signals that elicits tumor-specific cytotoxic T lymphocyte (CTL) responses. The immune effectors that are generated in this setting can act systemically, hence eradicating distant, non-irradiated lesions (long-range, out-of-field or abscopal effect). In immunocompetent hosts, the efficacy of radiotherapy appears to rely for the most part on abscopal effects. DC, dendritic cell.

autophagy has been suggested to constitute a valid strategy to circumvent the resistance of neoplastic cells to chemo- and radiotherapy.[25]

In a recent issue of *Cell Death and Differentiation*, Ko and colleagues investigated how the genetic inhibition of autophagy would impact on the response of human and murine lung carcinoma cells to radiation therapy, in vitro and in vivo.[26] In line with previous reports, these authors observed that cancer cells stably depleted of essential autophagic factors such as ATG5 and Beclin 1 are more sensitive to the cytostatic/cytotoxic effects of ionizing irradiation than their wild-type counterparts, in vitro. Along similar lines, autophagy-deficient cancer cells growing in immunodeficient hosts exhibited an improved response to radiation therapy as compared with autophagy-proficient cells of the same type.[26] These findings confirm that autophagy limits the efficacy of ionizing irradiation in settings in which therapeutic responses rely for the most part on cancer cell-intrinsic mechanisms.

Importantly, the response to radiotherapy of autophagy-incompetent tumors growing in syngeneic immunocompetent mice was decreased as compared with that of autophagy-competent neoplasms.[26] In this setting, the pharmacological inhibition of extracellular nucleotidases (artificially increasing the pericellular concentration of ATP) restored the radiosensitivity of autophagy-deficient cancers, hence facilitating tumor infiltration by lymphocytes. Thus, in scenarios in which radiotherapy exerts antineoplastic effects mostly through the activation of tumor-specific immune responses, autophagy mediates a beneficial, rather than detrimental, function.

Altogether, these observations indicate that in immunocompetent hosts, cancer cell-extrinsic, immunological mechanisms may contribute to the therapeutic efficacy of ionizing irradiation more significantly than cancer cell-intrinsic ones. In this setting, the use of pharmacological inhibitors of autophagy as a strategy to sensitize cancer cells to therapy may not be justified. Conversely, the combination of ionizing irradiation with agents that stimulate the autophagic flux and/or with immunostimulatory interventions might yield optimal therapeutic responses. Further investigation is required to address this hypothesis in detail.

References

1. Thariat J, Hannoun-Levi JM, Sun Myint A, Vuong T, Gérard JP. Past, present, and future of radiotherapy for the benefit of patients. Nat Rev Clin Oncol 2013; 10:52-60; PMID:23183635; http://dx.doi.org/10.1038/nrclinonc.2012.203
2. Vacchelli E, Prada N, Kepp O, Galluzzi L. Current trends of anticancer immunochemotherapy. Oncoimmunology 2013; 2:e25396; PMID:23894726; http://dx.doi.org/10.4161/onci.25396
3. Vacchelli E, Vitale I, Tartour E, Eggermont A, Sautes-Fridman C, Galon J, et al. Trial Watch: Anticancer radioimmunotherapy. OncoImmunology 2013; 2:e24238; http://dx.doi.org/10.4161/onci.24238
4. Stone HB, Coleman CN, Anscher MS, McBride WH. Effects of radiation on normal tissue: consequences and mechanisms. Lancet Oncol 2003; 4:529-36; PMID:12965273; http://dx.doi.org/10.1016/S1470-2045(03)01191-4
5. Travis LB, Ng AK, Allan JM, Pui CH, Kennedy AR, Xu XG, Purdy JA, Applegate K, Yahalom J, Constine LS, et al. Second malignant neoplasms and cardiovascular disease following radiotherapy. J Natl Cancer Inst 2012; 104:357-70; PMID:22312134; http://dx.doi.org/10.1093/jnci/djr533
6. Vitale I, Galluzzi L, Castedo M, Kroemer G. Mitotic catastrophe: a mechanism for avoiding genomic instability. Nat Rev Mol Cell Biol 2011; 12:385-92; PMID:21527953; http://dx.doi.org/10.1038/nrm3115

7. Galluzzi L, Kepp O, Kroemer G. Mitochondria: master regulators of danger signalling. Nat Rev Mol Cell Biol 2012; 13:780-8; PMID:23175281; http://dx.doi.org/10.1038/nrm3479
8. Mothersill C, Seymour CB. Radiation-induced bystander effects--implications for cancer. Nat Rev Cancer 2004; 4:158-64; PMID:14964312; http://dx.doi.org/10.1038/nrc1277
9. Prise KM, O'Sullivan JM. Radiation-induced bystander signalling in cancer therapy. Nat Rev Cancer 2009; 9:351-60; PMID:19377507; http://dx.doi.org/10.1038/nrc2603
10. Kaminski JM, Shinohara E, Summers JB, Niermann KJ, Morimoto A, Brousal J. The controversial abscopal effect. Cancer Treat Rev 2005; 31:159-72; PMID:15923088; http://dx.doi.org/10.1016/j.ctrv.2005.03.004
11. Kroemer G, Zitvogel L. Abscopal but desirable: The contribution of immune responses to the efficacy of radiotherapy. Oncoimmunology 2012; 1:407-8; PMID:22754758; http://dx.doi.org/10.4161/onci.20074
12. Lerret NM, Marzo AL. Adoptive T-cell transfer combined with a single low dose of total body irradiation eradicates breast tumors. Oncoimmunology 2013; 2:e22731; PMID:23525138; http://dx.doi.org/10.4161/onci.22731
13. Kepp O, Galluzzi L, Martins I, Schlemmer F, Adjemian S, Michaud M, Sukkurwala AQ, Menger L, Zitvogel L, Kroemer G. Molecular determinants of immunogenic cell death elicited by anticancer chemotherapy. Cancer Metastasis Rev 2011; 30:61-9; PMID:21249425; http://dx.doi.org/10.1007/s10555-011-9273-4
14. Kroemer G, Galluzzi L, Kepp O, Zitvogel L. Immunogenic cell death in cancer therapy. Annu Rev Immunol 2013; 31:51-72; PMID:23157435; http://dx.doi.org/10.1146/annurev-immunol-032712-100008
15. Ma Y, Adjemian S, Mattarollo SR, Yamazaki T, Aymeric L, Yang H, Portela Catani JP, Hannani D, Duret H, Steegh K, et al. Anticancer chemotherapy-induced intratumoral recruitment and differentiation of antigen-presenting cells. Immunity 2013; 38:729-41; PMID:23562161; http://dx.doi.org/10.1016/j.immuni.2013.03.003
16. Chekeni FB, Elliott MR, Sandilos JK, Walk SF, Kinchen JM, Lazarowski ER, Armstrong AJ, Penuela S, Laird DW, Salvesen GS, et al. Pannexin 1 channels mediate 'find-me' signal release and membrane permeability during apoptosis. Nature 2010; 467:863-7; PMID:20944749; http://dx.doi.org/10.1038/nature09413
17. Ma Y, Adjemian S, Yang H, Catani JP, Hannani D, Martins I, Michaud M, Kepp O, Sukkurwala AQ, Vacchelli E, et al. ATP-dependent recruitment, survival and differentiation of dendritic cell precursors in the tumor bed after anticancer chemotherapy. Oncoimmunology 2013; 2:e24568; PMID:23894718; http://dx.doi.org/10.4161/onci.24568
18. Panaretakis T, Kepp O, Brockmeier U, Tesniere A, Bjorklund AC, Chapman DC, Durchschlag M, Joza N, Pierron G, van Endert P, et al. Mechanisms of pre-apoptotic calreticulin exposure in immunogenic cell death. EMBO J 2009; 28:578-90; PMID:19165151; http://dx.doi.org/10.1038/emboj.2009.1
19. Ma Y, Galluzzi L, Zitvogel L, Kroemer G. Autophagy and cellular immune responses. Immunity 2013; 39:211-27; PMID:23973220; http://dx.doi.org/10.1016/j.immuni.2013.07.017
20. Michaud M, Martins I, Sukkurwala AQ, Adjemian S, Ma Y, Pellegatti P, Shen S, Kepp O, Scoazec M, Mignot G, et al. Autophagy-dependent anticancer immune responses induced by chemotherapeutic agents in mice. Science 2011; 334:1573-7; PMID:22174255; http://dx.doi.org/10.1126/science.1208347
21. Martins I, Wang Y, Michaud M, Ma Y, Sukkurwala AQ, Shen S, Kepp O, Métivier D, Galluzzi L, Perfettini JL, et al. Molecular mechanisms of ATP secretion during immunogenic cell death. Cell Death Differ 2013; forthcoming; PMID:23852373; http://dx.doi.org/10.1038/cdd.2013.75
22. Michaud M, Sukkurwala AQ, Martins I, Shen S, Zitvogel L, Kroemer G. Subversion of the chemotherapy-induced anticancer immune response by the ecto-ATPase CD39. Oncoimmunology 2012; 1:393-5; PMID:22737627; http://dx.doi.org/10.4161/onci.19070
23. Green DR, Galluzzi L, Kroemer G. Mitochondria and the autophagy-inflammation-cell death axis in organismal aging. Science 2011; 333:1109-12; PMID:21868666; http://dx.doi.org/10.1126/science.1201940
24. Kroemer G, Mariño G, Levine B. Autophagy and the integrated stress response. Mol Cell 2010; 40:280-93; PMID:20965422; http://dx.doi.org/10.1016/j.molcel.2010.09.023
25. White E. Deconvoluting the context-dependent role for autophagy in cancer. Nat Rev Cancer 2012; 12:401-10; PMID:22534666; http://dx.doi.org/10.1038/nrc3262
26. Ko A, Kanehisa A, Martins I, Senovilla L, Chargari C, Dugue D, Mariño G, Kepp O, Michaud M, Perfettini JL, et al. Autophagy inhibition radiosensitizes in vitro, yet reduces radioresponses in vivo due to deficient immunogenic signalling. Cell Death Differ 2013; In press; PMID:24037090; http://dx.doi.org/10.1038/cdd.2013.124.