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Functional Assays for Individual Radiosensitivity: A Critical Review

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A complete understanding of the mechanisms of the response to radiation would help in a better evaluation of the radiation-induced risks. To this aim, individual radiosensitivity, that is, the proneness to radiation-induced tissue reactions attributable to cell death, has been documented since the beginning of the 20th century. For several decades, developing informative predictive assays has been one of the most important challenges of radiobiologists. This article is a critical review devoted to the major functional assays to predict radiosensitivity and their strengths and weaknesses, notably those based on the quantification of clonogenic cell survival, micronuclei, p21 expression, apoptosis, chromosome and DNA repair, and signaling. Genomic approaches of radiosensitivity are reviewed in another article of this issue.

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Introduction

Less than 1 year after the discovery of X-rays by Roentgen,[1] Voigt described the first radiation-induced (RI) cutaneous reactions.[2] Thereafter, RI tissue reactions concerning other parts of the body were pointed out progressively.[3] At the beginning of the 20th century, the pioneers of radiation were aware that these RI tissue reactions were attributable to cell death[4,5] due to radiation dose excess or individual radiosensitivity.[6-8]

The first RI cancer was described by Frieben in 1902.[9] Between 1917 and 1926, the radium dial painters currently called "*the radium girls*," represented the first cohort of RI cancer cases.[10] This period coincided with the first international congress of radiology. Although the pioneers of radiation initially devoted the term "radiosensitivity" to RI tissue reactions, this term has also been used to describe RI cancer proneness, (Britel et al, submitted for publication) but the confusion between these 2 notions must be avoided.[13]

To date, there is increasing evidence that RI tissue reactions and cancers are not necessarily caused by the same molecular mechanisms. For example, the Li-Fraumeni syndrome caused by heterozygous *p53* mutations is associated with high cancer risk but not with severe RI tissue reactions.[11] Conversely, ataxia telangiectasia (AT) caused by homozygous *ATM* mutations is associated with both postradiotherapy fatal reaction and high lymphoma/leukemia risk.[12] Hence, we proposed to keep the historical meaning of the term "radiosensitivity" (ie, the proneness to RI tissue reactions attributable to cell death), and to use "radiosusceptibility" to describe the proneness to RI cancer, more likely attributable to cell transformation.[13]

Individual radiosensitivity is a critical issue in radiotherapy (RT) whose reliability imposes some specific constraints:

1. Radiosensitivity is not an *all-or-none* but a *continuous* phenomenon that is associated with a large spectrum of tissue reactions ranging from simple burns to patient death.[14,15] Predictive assays should reflect this continuous spectrum of responses.
2. Occurrences of RI tissues reactions are *dose-dependent*. One of the most representative examples of this dose-dependence is the case of a young patient who suffered from *LIG4* mutations and who succumbed during RT against lymphoma: death occurred after the first RT sessions, whereas he did not elicit any tissue reactions before.[16,17] Predictive assays should provide data from irradiated cells.

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3. Radiosensitivity can be observed on different tissues. Predictive assays should therefore reflect the *tissue-dependence* of radiosensitivity by involving the most representative cellular model of RI tissue reactions.[13]
4. A *quantified* relationship between clinical, cellular, and molecular end points is required to consolidate the relevance of predictive assays.

This article is a critical review devoted to the major functional assays to predict radiosensitivity and their strengths and weaknesses. Genomic approaches of radiosensitivity are reviewed in another article in this issue.

Tissular Biomarkers of Radiosensitivity

RI tissue reactions are generally observed after RT at doses higher than 0.5 Gy in a context of accidental dose excess (dosimetry error) or individual radiosensitivity.[13] These reactions vary extensively: inflammations, infections, ulcerations, fibrosis, necrosis, etc. Early dermatitis during RT against breast cancers and late proctitis after RT against prostate cancers are among the most frequent.[18] RI reactions are usually classified into early and late. However, there is now evidence that their occurrence may cover a continuous period of time beginning from the first RT session and ending months or years after the last one.[19] Since the 1970s, AT has been considered as the most radiosensitive genetic syndrome.[20] To date, although one case of death after RT was reported recently,[21] fatal issues after RT are extremely rare as the specific clinical features of AT are detectable in the pediatric setting. Hence, the RI tissue reactions in RT affect *morbidity* rather than *lethality*.

To date, approximately 5%-20% patients show RI tissue reactions after RT.[22] Since 1979, a number of clinical classifications of RI tissue reactions have flourished in literature.[23] The Common Terminology Criteria for Adverse Events (CTCAE)[24] and Radiation Therapy Oncology Group (RTOG)[25] scales are the most extensively used. These 2 scales classify RI tissue reactions into 6 grades (grade 0: no event; grade 5: death), independently of the irradiated organs and the early/late nature of RI reactions.

RI inflammations and tissue reactions are the complex result of successive molecular and cellular events. Particularly, most early-induced cytokines are proinflammatory. Hence, the RI expression of some cytokines, mainly interleukin 6 or transforming growth factor beta, has long been considered to be predictive biomarkers of post-RT inflammation.[26,27] For example, some circulating cytokine levels appeared to be good predictive markers of specific post-RT adverse effects (generally of severity grade equal or higher than 2), notably for RT against lung cancer.[28,29] Recently, the expression of transforming growth factor beta observed at week 6 of RT was found to be correlated with pneumonitis in patients with non–small cell lung cancer treated with 3-dimensional conformal RT.[30] Conversely, the same end points seem not to be correlated with late fibrosis, raising the question of the tissue specificity of cytokine assays.[31]

Another question raised by the cytokine assays is that they reflect preinflammatory events that are downstream of the DNA damage repair and signaling ones: they may be positive for different molecular and genetic origins. Furthermore, the cytokine assays require sampling *during RT* and therefore data may be obtained too late to allow efficient countermeasures. Further investigations are needed to examine whether cytokine assays can be used practically as predictive assays for specific RT and tumor localizations.

Cellular Biomarkers of Radiosensitivity

There are 3 major cell death pathways observed after irradiation that unequally contribute to the global inactivation of cellular clonogenic potential: mitotic death, apoptosis, and senescence. Interestingly, these types of cell death were already described before the discovery of X-rays.[32]

Mitotic death results in the formation of irreversibly damaged chromosomal fragments (micronuclei) expulsed from the nucleus.[33] It is the most frequent type of RI death for proliferating cells.[34] The number of residual micronuclei has been correlated with RI clonogenic inactivation.[35]

Senescence results in an irreversible permanent G_1 arrest.[36] It is the most frequent RI death for quiescent cells, notably for doses higher than 4 Gy. One of the most reliable end point of senescence is the expression of CDKN1A/p21. Postirradiation expression was shown to be significantly reduced for CDKN1A/p21 in radiosensitive patients with breast cancer. However, because of an overlap between radioresistant and radiosensitive patients, it was not possible to predict a normal tissue response. For example, by using a cutoff of 7-fold CDKN1A/p21 expression increase, a radiosensitive status was identified in approximately 91% of the patients tested.[37] Nevertheless, the relevance of the CDKN1A/p21 expression assay remains to be validated for a larger spectrum of radiosensitivity cases.

Apoptosis is one of the most documented death pathways although it is one of the rarest: apoptosis is mainly observed in lymphocytes and very rarely in fibroblasts. As AT (*ATM*-mutated) and Li-Fraumeni (*p53*-mutated) cells do not show apoptosis while they are hyperradiosensitive and radioresistant, respectively,[11] apoptosis alone cannot be a reliable predictor of radiosensitivity. Besides, there is still no general correlation between cellular radiosensitivity and apoptosis.[38,39] An inverse correlation between apoptosis and RI tissue reactions was observed after 8 Gy in lymphocytes of hundreds of patients on RT, such that *the lower the apoptosis yield the higher the radiosensitivity*.[40-42] Another study performed at 2 Gy indicated that *the higher the apoptosis yield the higher the radiosensitivity*.[43] This last study also showed that apoptosis, even when combined with some polymorphisms, can predict only acute dermatitis (10 cases) but not all of the other RI tissue reactions (84 cases) including late fibrosis in a cohort of patients with breast cancer.[43] Lastly, apoptosis assays in lymphocytes in a large series of patients with breast cancer failed to show any association with RI reactions.[44] The